Infection, Genetics and Evolution 40 (2016) 275–281

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Higher detection rates of amino acid substitutions in HBV reverse transcriptase/surface protein overlapping sequence is correlated with lower serum HBV DNA and HBsAg levels in HBeAg-positive chronic hepatitis B patients with subgenotype B2

Mingze Su, Kuanhui Xiang, Yao Li, Yutang Li, Juan Deng, Xizhan Xu, Ling Yan, Hui Zhuang *, Tong Li *

Department of Microbiology and Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, 38 Xueyuan Road, Haidian District, Beijing 100191, China

ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 7 March 2016
Accepted 18 March 2016
Available online 19 March 2016

Keywords:
Hepatitis B virus
Subgenotype B2
Reverse transcriptase
Surface protein
Substitution

ABSTRACT

Objectives: Hepatitis B virus (HBV) subgenotype B2 is prevalent in China and some other parts of Asia. This study aimed to carry out a subgenotype B2 specific mutation analysis on important amino acid (AA) sites in overlapping reverse transcriptase (RT) and surface (S) protein coding regions of HBV.
Materials and methods: A total of 143 hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB) patients with HBV subgenotype B2 infection were enrolled. HBV RT/S regions were sequenced focusing on 43 RT resistance AA sites and 31 S AA sites with functional/structural/conformational importance.
Results: According to the consensus AA sequence for subgenotype B2, 49.7% (71/143) of RT and 33.6% (48/143) of S protein sequences contained detectable substitutions at 58.1% (25/43) of studied AA sites in RT and 51.6% (16/31) of AA sites in S proteins, respectively. The most frequently detected substitutions were rtN134D/S (44/143, 30.8%) and sT126A/S (22/143, 15.4%), which were located in the RT A–B interdomain region and the corresponding antigenicity determinant region of S protein, respectively. In addition, two patients harboring drug resistance mutations rtL80V + rtM204I and rtL180M + rtM204V were found. Interestingly, the patients with detectable AA substitutions at any of the 74 sites in either/both of RT/S sequences had significantly lower serum HBV DNA and HBsAg levels than that in patients without detectable RT/S AA substitutions ($P < 0.05$). A trend Chi-squared test indicated that a negative association of serum HBsAg level with S protein sequence substitution rate was statistically significant ($P = 0.047$).
Conclusion: This subgenotype B2 specific mutation analysis revealed some naturally occurring hot spot substitutions at important AA sites of HBV RT/S proteins, which together might influence the serum HBV DNA and HBsAg levels in HBeAg-positive CHB patients.

1. Introduction

Hepatitis B virus (HBV) infection remains a serious global public health problem with about 350–400 million people infected worldwide (EASL, 2012). HBV has a partially double-stranded circular DNA genome with about 3215 base pairs and exhibits distinct genetic variability due to the lack of proofreading function of its DNA polymerase/reverse transcriptase (RT) (Li et al., 2013). A genetic variation beyond 7.5% of the HBV full-length genome sequence is defined as a genotype, which shows a geographic prevalence property. At present, HBV is classified into 10 genotypes (A–J) and more than 35 subgenotypes (Kramvis, 2014). In China, genotypes B and C are the most prevalent, with the former being more prevalent in southern part and the latter in northern part. In addition, most of B and C genotype strains are reported to be subgenotypes B2 and C2, respectively (Wang et al., 2007).

Point mutations have been frequently reported throughout the HBV genome sequences with various biological and clinical consequences (De Maddalena et al., 2007; Pollicino et al., 1997). For more than a decade, extensive mutation analyses have been focused on HBV RT region due to the widely clinical applications of nucleos(t)ide analogs (NAs) and the development of NA resistance (NAr) mutations in RT, such as rtM204I/V which causes resistance to lamivudine (LMV) and telbivudine (LdT) (Locarnini and Yuen, 2010). Several studies have focused on the concomitant mutations in RT and S regions since their protein coding regions overlap (Fig. 1) (Bartholomeusz et al., 2004;

Abbreviations: AA, amino acid; ADV, adefovir dipivoxil; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HSPGs, highly sulfated heparan sulfate proteoglycans; LdT, telbivudine; LMV, lamivudine; MHR, major hydrophilic region; NAs, nucleos(t)ide analogs; NAr, nucleos(t)ide analog resistance; RT, reverse transcriptase; S, surface.

* Corresponding authors.
E-mail addresses: zhuangbmu@126.com (H. Zhuang), tongli08@vip.sina.com (T. Li).

http://dx.doi.org/10.1016/j.meegid.2016.03.019
1567-1348/

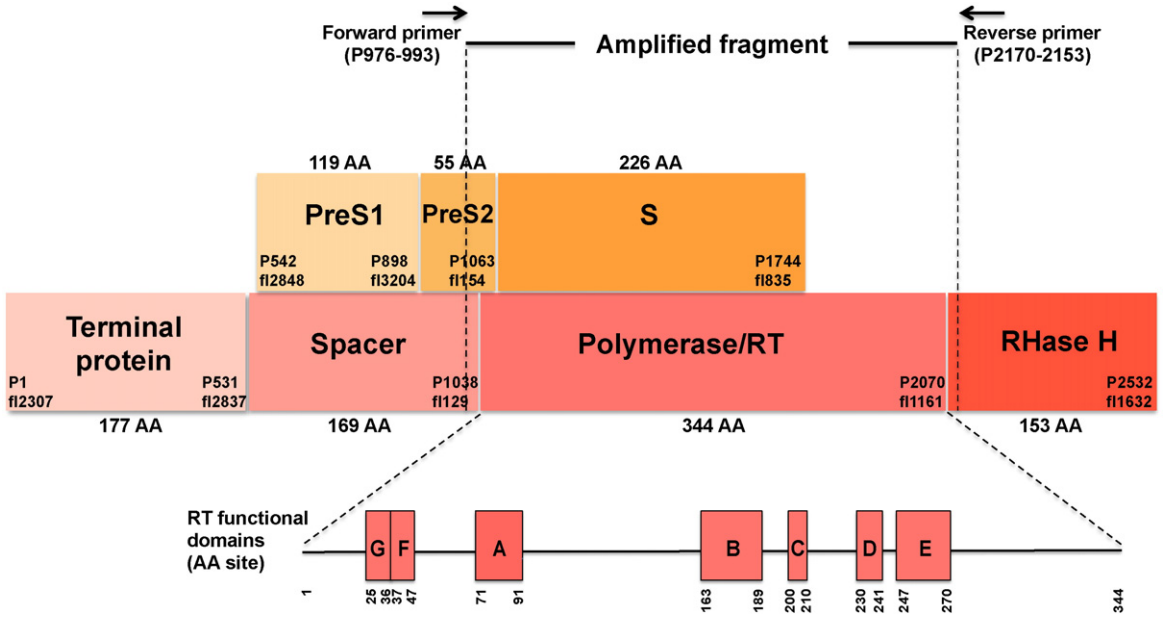

Fig. 1. Schematic diagram of HBV S and P gene structure showing the overlapping feature of RT and S coding regions. Numbering was according to genotype B and C HBV nucleotide and AA sequences (Bartholomeusz et al., 2004; Clark and Hu, 2015; Hao et al., 2015). AA, amino acid; fl, full length; P, polymerase; RT, reverse transcriptase; S, surface.

Locarnini and Yuen, 2010; Teo and Locarnini, 2010; Clark and Hu, 2015; Hao et al., 2015). The RT activity is important for the replication of HBV DNA and the expression of S protein is essential for the virion assembly and secretion. In clinical settings, serum HBV DNA and hepatitis B surface antigen (HBsAg) levels are virological markers reflecting viral replication. Therefore, they are used to define the course for antiviral treatment and to predict treatment responses in chronic hepatitis B (CHB) patient (Lesmana et al., 2014). However, the impact of HBV subgenotypes on RT/S sequences and subsequently on serum HBV DNA and HBsAg levels have not been well characterized, especially in the case of subgenotype B2. To our surprise, a PubMed search for "HBV subgenotype and mutation" and "HBV subgenotype and substitution" as key words showed only 97 and 22 hits respectively (access on December 7, 2015). Only a few of those studies focused on HBV S protein AA substitution analyses mainly on the major hydrophilic region (MHR) of HBsAg in genotype D HBV isolates (Kitab et al., 2011; Pourkarim et al., 2014). One should remember that the genetic makeup still has an up to 4.0% difference within a HBV subgenotype, which is large enough to influence the virus replication, antigen production and clinical consequence of the disease (Kramvis, 2014). In a study, which took HBV subgenotype into account, Lyoo et al. reported that AA substitutions in immune epitopes of hepatitis B core antigen of genotype C isolates were significantly associated with disease progression (Lyoo et al., 2011). The naturally occurring AA substitutions within HBV RT/S regions and their potential clinical significance certainly call for more attention.

In this study, we carried out a subgenotype-specific mutation analysis both on RT and its overlapping S gene regions. The enrolled 143 CHB patients were hepatitis B e antigen (HBeAg) positive with defined B2 subgenotype HBV infection without antiviral treatment at least six months prior to enrollment. The mutation analysis focused on 43 NAr-related RT residues, which we have previously described (Liu et al., 2010; Zhao et al., 2012), and 31 conformation- and antigenicity-related S protein residues (Table 1) (Ding et al., 2014). The resulting consensus RT and S protein sequences and the identified naturally occurring AA substitution patterns as well as the potential clinical significance would provide valuable information for a better understanding of B2 subgenotype mutations in this important RT/S region.

2. Materials and methods

2.1. Patients

This study was approved by the Ethics Committee of Peking University Health Science Center, and an informed consent was obtained from each patient. The serum samples were obtained from HBeAg-positive CHB patients from 23 hospitals in China from Match 2010 to Match 2013. Only those identified with HBV subgenotype B2 infection (143 patients) were enrolled in this study (see subgenotyping method in

Table 1
Consensus AA sequence residues of HBV RT and S protein of subgenotype B2 were analyzed in this study.

Category of analyzed AA sites (no.)	Consensus AA in B2 subgenotype
RT region (43[a])	
RT-1. Primary NAr AA sites (8)	rtI169 rtA181 rtT184 rtA194 rtS202 rtM204 rtN236 rtM250
RT-2. Secondary NAr AA sites (3)	rtL80 rtV173 rtL180
RT-3. Putative NAr-associated AA sites (27)	rtN53 rtT54 rtR55 rtL82 rtV84 rtS85 rtL91 rtH126 rtT128 rtN139 rtR153 rtF166 rtV191 rtA200 rtV207 rtS213 rtV214 rtQ215 rtL217 rtE218 rtY221 rtL229 rtI233 rtP237 rtH238 rtY245 rtS256
RT-4. Pretreatment NAr-associated AA sites (6)	rtT38 rtN124 rtN134 rtN139 rtV224 rtR242
S region (31)	
S-1. Sero-subtype-associated AA sites (7)	sK122 sP127 sF134 sA159 sK160 sV177 sP178
S-2. Disulfide-bridge-cysteine pairs (8)[b]	sC107–sC138 sC121–sC124 sC137–sC149 sC139–sC147
S-3. Immune escape AA sites (10)	sP120 sT126 sQ129 sG130 sT131 sM133 sK141 sT143 sD144 sG145
S-4. NAr-associated mirror change AA sites (6)	sE164 sW172 sL173 sW182 sI195 sW196

AA, amino acid; NAr, nucleos(t)ide analog resistance; RT, reverse transcriptase; S, surface.
[a] Categories RT-3 and RT-4 shared rtN139 site due to a difference in detected substitution types either during antiviral treatment (category RT-3, rtN139D) or at treatment baseline (category RT-4, rtN139K/H) (Liu et al., 2010). Thus, altogether 43 sites in the RT region were analyzed.
[b] The consecutive two AA sites formed a disulfide-bridge-cysteine pair.