Hepatitis delta virus facilitates the selection of hepatitis B virus mutants *in vivo* and functionally impacts on their replicative capacity *in vitro*

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Abstract

To identify molecular interactions between hepatitis B virus (HBV) and hepatitis delta virus (HDV), HBV sequences were analysed in HBV/HDV-infected patients. Characteristic amino acid substitutions were found in cytosolic domains of hepatitis B surface antigen (HBsAg), in contrast to HBV-mono-infected controls. The functional impact of HDV on the replication of wild-type and mutant HBV was assessed *in vitro*. HDV co-transfection significantly reduced the replication of HBV strains containing precore or basal core promoter mutations, and HBV polymerase or surface antigen mutants affected HDV replication *in vitro*. Conclusively, our study revealed distinct HBsAg mutational patterns in HBV/HDV-infected patients and novel functional interactions between HBV and HDV.

Keywords: Basal core promoter, HBsAg, hepatitis B virus, hepatitis delta virus, precore
Original Submission: 4 May 2015; **Revised Submission:** 7 September 2015; **Accepted:** 19 September 2015
Editor: G. Antonelli
Article published online: 30 October 2015

Hepatitis delta virus (HDV) co-infection or superinfection in hepatitis B virus (HBV)-infected individuals is the most severe form of chronic hepatitis. Although HBV DNA is often suppressed in co-infected patients, fluctuating or persistently high levels of HBV viraemia occur in delta hepatitis [1,2]. We recently reported the distinct mutational pattern in the HDV genome from a nationwide Iranian cohort, where the prevalence of HDV is high [3]. We have now analysed the impact of HDV infection on the genomic sequences and functional properties of HBV in double-infected individuals. We studied HBV sequences of Small hepatitis B surface antigen (S-HBsAg), reverse transcriptase (rt), transcription regulator (including EnhI, EnhII, and Box-β), basal core promoter (BCP) and precore (PC) domains from HBV/HDV-infected patients with amplified HBV DNA in a case–control setting. For each case with amplified HBV in HBV/HDV-infected patients, two HBV-mono-infected patients matched for sex, age, genotype and hepatitis B e antigen (HBeAg) status were studied as controls. The HBV/HDV-infected patients more frequently had cirrhosis than HBV-mono-infected individuals, whereas their HBV DNA levels were lower (Table 1). Owing to reduced

TABLE 1. Demographic, clinical and virological data of the patients

	HBV control group	HBV/HDV case group	p
Total patients (*n*)	NA	71	NA
Enrolled patients (*n*)	34	17	NA
Female, *n* (%)/male, *n* (%)	14 (41.1)/20 (58.8)	7 (41.1)/10 (58.8)	NS
Age (years)	37.4 (17–54)	38.5 (18–58)	NS
Cirrhosis (%)	14.7	52.9	<0.001
ALT (U/L), median (range)	49 (15–172)	48 (20–86)	NS
HBV viral load (copies/mL), median (range)	6.8×10^5 (3.1×10^3 to 8.3×10^8)	4.6×10^3 (1.1×10^3 to 2.3×10^5)	<0.001
HBeAg status	Negative	Negative	NS
Viral genotype			
HBV	D	D	NA
HDV	NA	I	NA
Treatment (history)			
IFN (%)	26	23	NS
Nucleot(s)ide analogues (%)	62	59	NS
No treatment (%)	12	18	NS

From 71 studied patients with positive hepatitis D antigen antibody, 24 had detectable HBV DNA in serum by real-time PCR. Of this group, 17 cases were successfully amplified and sequenced by in-house PCR. Each HBV/HDV-infected patient was matched with two HBV-mono-infected control patients on the basis of age, sex, viral genotype, and HBeAg negativity. Differences were considered to be significant if the p-value was <0.05 (chi-square or Mann–Whitney *U*-test).
ALT, alanine transaminase; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HDV, hepatitis delta virus; IFN, interferon; NA, not applicable; NS, not significant.

http://dx.doi.org/10.1016/j.cmi.2015.09.020

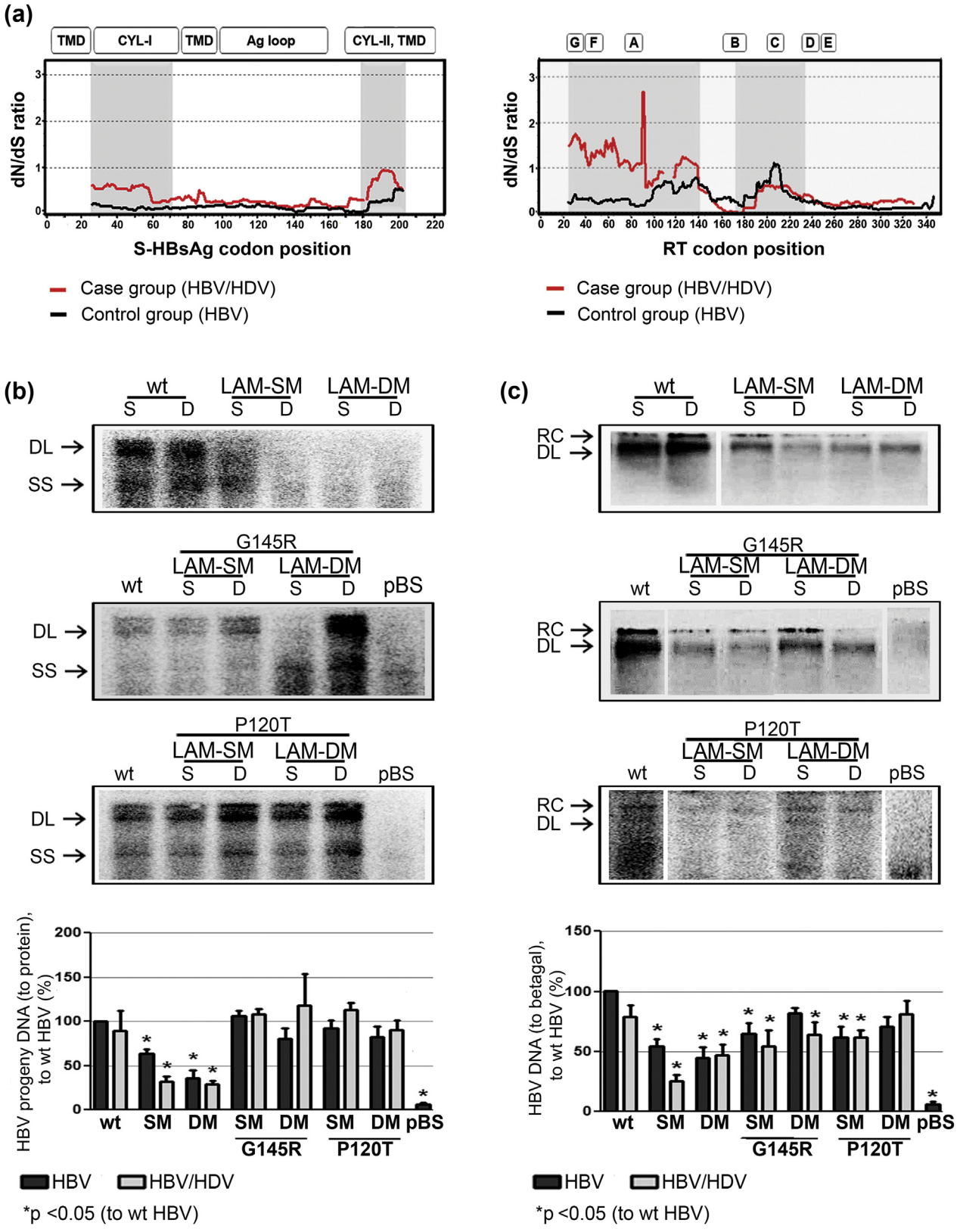

FIG. 1. Hepatitis B virus (HBV) genome alterations in HBV/hepatitis delta virus (HDV)-infected patients and functional impact of HDV on the replication of HBV polymerase and envelope mutants. (a) Comparison of amino acid substitution rates (dN/dS) for the small hepatitis B surface antigen