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Evaluation of Amino Acid Substitutions in the Overlapping Envelope and Polymerase Genes of HBV Among Treatment Naïve and Experienced Subjects Reveal Significant Changes Associated with Lamivudine and Adefovir, but not Tenofovir DF Treatment

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Background

- The hepatitis B virus (HBV) is composed of an overlapping reading frame. As a consequence, mutations in the envelope (HBsAg) gene may produce changes in the overlapping polymerase (pol/RT) gene. Similarly, mutations in pol/RT may produce changes in HBsAg (Figure 1)
- During long-term therapy with oral anti-HBV agents (lamivudine, adefovir dipivoxil, entecavir, and telbivudine) mutations not only occur in HBV pol/RT, but also in HBsAg resulting in reduced antigenicity of the HBsAg protein[1-3]
- 2 types of HBsAg mutants are recognized:
  - Those that arise as a result of amino acid substitutions caused by primary and compensatory resistance mutations in pol/RT
  - Those that arise because of prolonged viral suppression leading to seroclearance of HBV surface antigen, where vaccine-escape-like mutants might be selected

Objective

- To evaluate amino acid changes within the HBsAg reading frame from treatment-naïve, lamivudine (LAM)-, adefovir dipivoxil (ADV)-, ADV+LAM-, and tenofovir DF (TDF)-experienced subjects and determine if significant differences exist between treatment-experienced subjects as compared to treatment-naïve subjects
- To evaluate if established pol/RT LAM and/or ADV resistance mutations impact the amino acid sequence in the overlapping HBsAg reading frame

Methods

- Subjects were enrolled in one of 4 double-blind, randomized studies of TDF [Study 102 (HBeAg-), Study 103 (HBeAg+), Study 121 (LAM-R) and Study 106 (ADV+/-LAM-exp)]
- Subjects were categorized into 5 mutually exclusive groups; treatment-naïve, LAM-, ADV-, LAM+ADV-, and TDF-experienced
- Genotypic analysis of serum isolates by population di-deoxy sequencing
  - Covers AA 1-344 of pol/RT and AA 1-226 of HBsAg
  - Detects AA substitutions present at ≥ 25% of viral quasi-species population
  - Viral load limit is ≥ 400 copies/mL (2.6 $\log_{10}$ copies/mL, 69 IU/mL)
- AA frequency at each position in HBsAg was compared between treatment-naïve and treatment-experienced subjects using a Fisher's exact test with a significance threshold of p ≤0.01
- Criteria for HBsAg conserved sites; based on data from treatment-naïve subjects
  - 100% conserved
  - ≤ 3 AA possibilities with ≥ 99% homology at any given site

Table 1. Subject Characteristics

Category	Treatment Naïve (N=422)	LAM-exp (N= 269)	ADV-exp (N=122)	LAM-exp + ADV-exp (N=63)	TDF-exp (N=48)
Median HBV DNA $\log_{10}$ copies/mL (Min, Max)	7.64 (2.23, 10.92)	6.63 (2.99, 10.12)	5.07 (2.23, 9.57)	5.81 (3.09, 9.47)	3.78 (2.62, 9.07)
Median ALT IU/mL (Min, Max)	107 (23, 964)	49 (8,1302)	33.5 (13, 281)	49 (16, 816)	44.5 (15, 245)
HBeAg Positive, n (%)	160 (37.9%)	132 (49.1%)	66 (54.1%)	49 (77.8%)	38 (79.2%)
HBeAg Negative, n (%)	262 (62.1%)	137 (50.9%)	20 (16.4%)	14 (22.2%)	10 (20.8%)
HBeAg Data Missing	0 (0%)	0 (0%)	36 (29.5%)	0 (0%)	0 (0%)
Treatment Exposure (Years) (Min, Max)	NA	3.2 (0.1, 15.9)	0.9 (0.5, 2.5)	2.7 (0.7, 8.8)	1.5 (0.6, 2.9)
Viral Genotype, n (%)					
A	72 (17.1%)	59 (21.9%)	19 (15.6%)	16 (25.4%)	5 (10.4%)
B	57 (13.5%)	37 (13.8%)	14 (11.5%)	3 (4.8%)	4 (8.3%)
C	76 (18%)	52 (19.3%)	30 (24.6%)	12 (19.0%)	9 (18.8%)
D	198 (46.9%)	119 (44.2%)	56 (45.9%)	26 (41.3%)	28 (58.3%)
E-H/Missing	19 (4.5%)	2 (0.7%)	3 (2.5%)	6 (9.5%)	2 (4.2%)

Results

Figure 1. The Hepatitis B Virus is Composed of an Overlapping Reading Frame

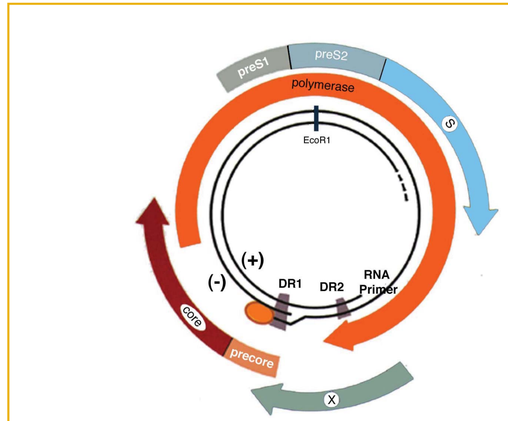


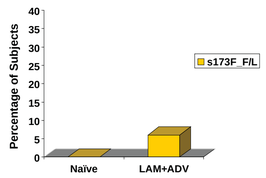
Table 2. Sequence Variation at Thirty Sites Within HBsAg Were Significantly Associated with Treatment Experience

HBsAg Position	HBsAg Conserved or Polymorphic	pol/RT Position	Treatment	p value
8	Polymorphic	16	LAM+ADV	0.009
30	Polymorphic	38	LAM+ADV	0.009
40	Polymorphic	48	ADV	0.011
56	Polymorphic	64	LAM+ADV	0.013
76	Polymorphic	84	LAM	0.007
110	Polymorphic	118	LAM	0.004
125	Polymorphic	133	LAM	0.008
126	Polymorphic	134	LAM	0.009
131	Polymorphic	139	TDF	0.010
134	Polymorphic	142	LAM	0.004
140	Polymorphic	148	LAM	0.007
164	Polymorphic	173	LAM	0.003
172	Conserved	181	LAM, ADV and LAM+ADV	0.009, <0.001, and 0.002
173	Conserved	181	LAM+ADV	<0.001
175	Conserved	184	LAM	0.009
184	Polymorphic	192	LAM+ADV	0.007
192	Conserved	200	LAM	0.003
195	Conserved	204	LAM and LAM+ADV	both <0.001
196	Conserved	204	LAM	<0.001
198	Polymorphic	206	LAM and ADV	<0.001 and 0.011
199	Polymorphic	207	LAM	0.009
204	Polymorphic	212	LAM and LAM+ADV	0.001 and 0.007
206	Polymorphic	214	LAM+ADV	0.009
207	Polymorphic	215	LAM and TDF	0.005 and 0.006
208	Polymorphic	216	LAM	<0.001
210	Polymorphic	218	LAM	<0.001
213	Polymorphic	221	LAM and LAM+ADV	0.006 and 0.012
217	Polymorphic	225	LAM	0.009
220	Polymorphic	228	LAM	<0.001
221	Polymorphic	229	LAM	0.004

Orange = HBsAg conserved site significantly associated with LAM and/or ADV resistance
Green = HBsAg polymorphic site significantly associated with LAM resistance

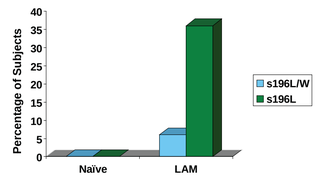
Figure 2. Sequence Variation at Six Conserved Sites in HBsAg Significantly Associated* with LAM and/or ADV Treatment, but not TDF Treatment

Figure 2A. HBsAg Sites Associated with One Treatment



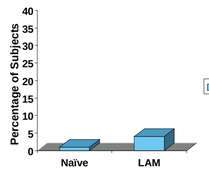
Associated with Primary ADV Resistance

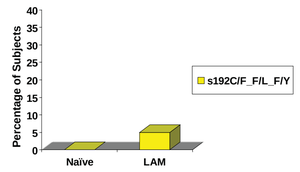
sL173L = rtA181A
sL173F = rtA181V



Associated with Primary LAM Resistance

sW196W = rtM204M
sW196L = rtM204I



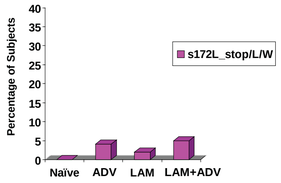


Sites Associated with Compensatory LAM Resistance

sL175L = rtT184T
sL175S = rtT184T
sL175F = rtT184S

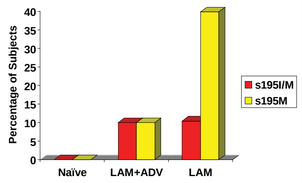
sL192L = rtA200A
sL192C = rtA200A
sL192F = rtA200V
sL192Y = rtA200V

Figure 2B. HBsAg Site Associated with Multiple Treatments



Associated with Long Term LAM and/or ADV Treatment

sW172W = rtA181A
sW172L = rtA181A
sW172stop = rtA181T



Associated with Primary LAM Resistance

sI195I = rtM204M
sI195M = rtM204V

*Statistically significant compared to treatment naïve group (p≤ 0.01)

Results Summary

- Thirty distinct sites in HBsAg was significantly associated with treatment experience (Table 2)
  - 6 distinct conserved HBsAg sites were significantly associated with treatment experience; none were associated with TDF
  - 24 distinct polymorphic HBsAg sites were significantly associated with treatment experience; two were associated with TDF
- No amino acid changes in HBsAg were associated with known vaccine escape mutants (sP120T, sT123N, sD144E, and sG145R) (Table 2)
- All six HBsAg conserved sites corresponded with known LAM and/or ADV resistance mutations in pol/RT (Figure 2A-2B)
- Sequence variation at one polymorphic HBsAg site significantly associated with treatment corresponded to a known LAM compensatory resistance mutation in pol/RT (sE164D/rtV173L) (Table 2)

Conclusions

- **Variation at 6 HBsAg amino acid positions was found to be significantly associated with LAM and/or ADV-treatment, but not TDF-treatment**
  - **HBsAg amino acid changes corresponded to sites in HBV pol/RT associated with LAM and ADV resistance**
- **Changes at HBsAg polymorphic sites were frequently associated with LAM-treatment**
- **No HBsAg amino acid changes were associated with vaccine escape mutants**

References

1. Torresi et al. 2002. Virology 293: 305-313
2. Torresi et al. 2002. Virology 299: 88-99
3. Sloan et al. 2008. Antiviral Therapy 13: 439-447