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FULL GENOME ULTRA-DEEP PYROSEQUENCING IDENTIFIES GG-TO-GA HYPERMUTATION AS NOVEL PREDICTOR FOR TREATMENT RESPONSE TO PEGYLATED INTERFERON ALFA-2A IN HBEAG-NEGATIVE CHRONIC HEPATITIS B

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Background and Aims: Interferon alfa and nucleos(t)ide analogues are the currently approved treatment options for chronic hepatitis B. The role of host genetic factors for response prediction is not conclusive.
Methods: Full genome ultra-deep pyrosequencing (UDPS) was performed on pre-treatment serum samples from 33 HBeAg-negative patients treated with pegylated interferon alfa-2a ± lamivudine. Sustained response was defined as hepatitis B virus (HBV) DNA level below 20,000 copies/mL combined with normalization of ALT (<30 international units/mL) at week 72. Various hypermutation rates were computed using the number of atypical G-to-A mutations and the dinucleotide preference GX-to-AX (X = A, C, G, T) statistics over a grid of cutoff values. Informative hypermutation criteria were identified using feature selection algorithms and treatment response was predicted with a statistical model. Twenty times ten-fold cross-validation was used to assess the prediction accuracy.
Results: Sustained response to peginterferon alfa-2a was achieved by eight of 33 patients. Correlating response and hypermutation rates revealed that the prediction performance strongly depends on the individual genomic region analysed for hypermutation rates. Best performance was achieved using only GG-to-AG hypermutation rates within nucleotide positions 500 to 1200 (count EcoRI restriction site = 0) together with baseline HBV DNA levels. We found that a high prevalence of GG-to-AG hypermutated sequences in combination with low HBV DNA levels is indicative of treatment response. The area under the receiver operating characteristic curve of this model was 0.89±0.03. The negative predictive value was 0.93±0.04%, positive predictive value 0.47±0.04% and accuracy amounted to 0.73±0.03%.
Conclusions: GG-to-AG hypermutations were identified as a novel predictor for sustained response to peginterferon alfa-2a by analysing pre-treatment UDPS data. As the GG dinucleotide is the preferred editing context of the cellular cytidine deaminase APOBEC3G, our findings suggest an important role of APOBEC3G in interferon induced virus control.

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EMERGENCE OF HBSAG ESCAPE MUTATIONS IS ASSOCIATED WITH USE OF NUCLEOS(T)IDE ANALOGUES IN CHRONIC HEPATITIS B PATIENTS

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Introduction: Chronic viral hepatitis B remains a global public health concern. HBV is a small DNA-containing virus with 4 genes (*C*, *S*, *X* and *P*). The *S* gene codes for the surface antigen (HBsAg), which contains the "a" determinant, the main region for induction of a protective humoral immune response. HBsAg escape mutation referred to amino acid substitution that can lead to emergence of immune-, detection- and vaccine escape mutants. There is little data on association of nucleos(t)ide analogue therapy with selection of HBsAg escape mutations. The aim of this study was to determine the association of nucleos(t)ide analogue therapy with emergence of HBsAg escape mutants.
Material and Methods: A cross-sectional study was performed on 80 patients with chronic hepatitis B under treatment of nucleos(t)ide analogues (Lamivudine and Adefovir) and 50 naive chronic hepatitis B patients. HBV DNA was extracted from plasma and S gene of virus was amplified by Nested-PCR Followed by direct sequencing. HBsAg amino acid sequence of samples were evaluated for detection of HBsAg escape mutations.
Results: Phylogenetic analysis showed that all (100%) of patients were infected with HBV genotype D. In the group of patients under treatment of nucleos(t)ide analogues, 27 (33.7%) patients infected with at least one HBsAg escape mutant strain versus 3 (6%) patients in the naive group ($P = 0.0003$, OR = 7.87, 95% CI = 2.44–35.54).
Conclusion: Our study revealed that HBV nucleos(t)ide analogue therapy can induce immune escape mutants in HBV genotype D infected patients. The important question is whether, these escape mutants can infect the previously vaccinated individuals, so further studies should be done.

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CLINICAL, VIROLOGICAL, SEROLOGICAL AND HISTOLOGICAL OUTCOMES IN CIRRHOTIC PATIENTS WITH CHRONIC HEPATITIS B (CHB) TREATED WITH TENOFOVIR DISOPROXIL FUMARATE (TDF) FOR UP TO 5 YEARS

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CHB suppression with TDF improves transaminases, results in a regression of liver fibrosis and increases HBeAg seroconversion. Long term data in cirrhotic patients is needed.
Methods: A retrospective analysis of 2 pivotal studies of TDF was conducted to assess whether cirrhotic patients had different clinical outcomes than non-cirrhotic patients. In these studies, the safety and efficacy of TDF compared to adefovir was assessed for 48 weeks in HBeAg negative (Study 0102) and HBeAg positive (Study 0103) patients, followed by open-label TDF treatment.

Table 1. Baseline characteristics of cirrhotics vs. non-cirrhotics

Parameter	Baseline cirrhotic (n = 152)	Baseline non-cirrhotic (n = 482)	P-Value
% >40 years old	68%	45%	<0.001
% male	81%	72%	0.022
Mean BMI (kg/m^2)	26.5	25.1	<0.001
Mean platelets ($\times 10^3$)	178	219	<0.001
Mean albumin (g/dL)	4.0	4.2	<0.001

Results: A total of 634 subjects were included. Cirrhotics had outcomes comparable to non-cirrhotics (see tables).
There were no cases of hepatic encephalopathy or variceal bleeding in either group, and ascites occurred in one non-cirrhotic patient with HCC. The incidence of HCC was 1.5% in non-cirrhotics and 3.3% in cirrhotics (p = NS).
Conclusions: Cirrhotic patients differed from non-cirrhotic subjects at baseline, but demonstrated significant treatment benefits with 74% experiencing regression of cirrhosis. Cirrhotic patients treated with TDF had similar rates of viral suppression and serological