Deep Sequencing shows that the presence of HBV BCP/PC variants reduces the rate of HBsAg loss among HBeAg-positive CHB patients treated with long-term TDF

Margaret Littlejohn
Victorian Infectious Diseases Reference Laboratory
Doherty Institute, Melbourne, Australia.
Disclosure:

This study was funded by Gilead Sciences
HBsAg seroconversion– functional cure

- HBsAg seroconversion is regarded as a “functional cure” and associated with a good prognosis.

- Seroclearance before the age of 50 appears to be important (Yuen et al 2008 Gastroenterology)

- The annual rate of HBsAg clearance has been estimated to be 0.5%-2% in Western patients, but only 0.1%-0.8% in Asian populations (Liaw et al 1991 Hepatology)
HBV is not a “one type fits all” disease

- 10 genotypes and multiple subgenotypes
- There are marked differences in:
  - Modes of transmission
  - Age of acquisition
  - Disease progression / outcome
  - Response to therapy (IFN and now NA)

- We are interested in identifying biomarkers predicting serological response to treatment
BCP mutations are associated with progressive liver disease

• BCP mutations are strongly associated with liver cirrhosis and HCC development.

• Ultradeep sequencing shows that patients harboring a high proportion of BCP mutants (≥45%) one year after HBeAg seroconversion have a higher likelihood of progressing to cirrhosis than those with a lower mutant percentage(<10%) (Tseng et al. Gut 2015;64:292-302)

• The reasons/mechanisms for this are unclear.

• The association of BCP mutations with HBsAg loss is yet to be investigated.
BCP/PC variants reduce/abolish HBeAg

PC variant = G1896A
- Translational stop codon
- Precore mRNA transcribed
- no HBeAg translated

BCP variant = A1762T/G1764A
- Reduces promoter activity
- Relative reduction in transcription activity
  - Reduced PC mRNA
  - Reduced HBeAg
Cohort

• GS-US-174-0103 was a randomised double-blind, phase 3 clinical trial which compared the efficacy of once-daily TDF or ADF monotherapy for 48 weeks against HBV.

• Upon completion of 48 weeks of TDF or ADF monotherapy all subjects received open-label TDF for an additional 336 weeks.

• All patients were nucleotide analogue naïve (or had limited lamivudine experience).

• Patients were classified as being in the immune clearance (IC) phase;
  • high viral load (HBV DNA > $10^6$ copies/mL),
  • hepatitis e antigen (HBeAg) positive
  • elevated alanine transaminase (ALT) (2-10X ULN).
Serological outcomes

• HBeAg seroconversion to anti-HBe:
  • 25% by week 48
  • 50% by week 192
  • 55% by week 336

• HBsAg loss (functional cure):
  • 11% by week 192
  • 11.8% by week 336 with 8% of patients seroconverted to anti-HBs.

• Almost all patients who experienced HBsAg loss (21/23) were infected with HBV genotypes A or D, with only one genotype B and no genotype C patients achieving HBsAg loss.

• HBsAg loss was associated with higher levels of hepatitis B virus (HBV) DNA in plasma and higher serum HBsAg titres at baseline.
Our primary area of interest was serological response

- HBeAg seroconversion by W192 (Year 4)
- HBsAg loss by W192

- We used Illumina Next Generation Sequencing to identify predictive biomarkers of serological response, particularly HBsAg loss.
- Sanger sequencing was performed for comparison
- We analysed baseline serum samples from 157 patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>35 (21%)</td>
<td>33 (19%)</td>
<td>54 (32%)</td>
<td>48 (28%)</td>
<td>157</td>
</tr>
</tbody>
</table>
NGS detected BCP and PC mutations with greater sensitivity than Sanger population sequencing
The frequency of BCP and PC mutations varied by genotype

Gt A (NGS, n=36)
- WT, 67%
- BCP, 28%
- BCP+PC, 3%

Gt B (NGS, n=24)
- WT, 0%
- BCP, 8%
- BCP+PC, 17%
- PC, 75%

Gt C (NGS, n=51)
- WT, 10%
- BCP+PC, 29%
- PC, 10%
- BCP, 51%

Gt D (NGS, n=46)
- WT, 33%
- BCP, 15%
- BCP+PC, 26%

High levels of WT in Gen A and D.

Almost no wildtype in Gen B and C!!

Gen B mostly PC
Gen C mostly BCP
BCP and PC variants were rarely detected in patients who subsequently lost HBsAg

NGS, n = 157

41% of these patients lost HBsAg

Only 3% of patients with these mutations lost HBsAg

<table>
<thead>
<tr>
<th></th>
<th>NPV (BCP/PC)</th>
<th>PPV (WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population seq</td>
<td>99%</td>
<td>21%</td>
</tr>
<tr>
<td>NGS</td>
<td>97%</td>
<td>47%</td>
</tr>
</tbody>
</table>
Other Factors

- Duration of infection may be important in likelihood of HBsAg loss.

- Genotypes A and D are almost always acquired by horizontal infection, in adulthood (A) or adolescence (D).

- Genotypes B and C are almost exclusively perinatal transmission, i.e., birth or very early childhood.

- However, multivariate analysis showed that it was presence of BCP mutations, not duration of infection that was associated with reduced HBsAg loss.
Viral diversity varied across the genome (determined by Shannon entropy)

Diversity was always higher in genotypes B/C than A/D.
Increased sequence diversity was associated with reduced likelihood of HBsAg loss (Gen A/D)
Mechanism?

- The basal core promoter regulates transcription of pgRNA and pcRNA, **NOT** HBsAg mRNAs

- But could they be altering cccDNA expression?
Overlapping nature of the HBV genome means BCP mutations also alter the HBx gene.

HBx is a transactivating protein which is required for HBV infection and plays a major role in regulating expression of the major HBV transcriptional template, covalently closed circular (ccc) DNA.

We are currently exploring the role of HBx mutants in regulating HBsAg production across genotypes.
Summary

• Presence of BCP/PC variants at baseline significantly reduces the likelihood of HBsAg loss in the setting of NA therapy in HBeAg positive individuals.

• Loss of HBsAg was observed in association with WT virus and reduced viral diversity.

• The relationship between BCP/PC variants and surface antigen is not yet clear.
Acknowledgements

Victorian Infectious Diseases Reference Laboratory (VIDRL)
Peter Revill
Julianne Bayliss
Gillian Rosenberg
Lilly Yuen
Darren Wong
Renae Walsh
Thao Huynh
Xin Li
Rachel Hammond
Danni Colledge
Nadia Warner
Ros Edwards
Kathy Jackson
Scott Bowden
Stephen Locarnini

St Vincent's Hospital
Alexander Thompson

Gilead Biosciences
Kathryn Kittrinos
Mani Subramanian
Anuj Gaggar

Studies
Clinicians
Participants

Disclosure: This study was funded by Gilead Biosciences
Next Gen Platform

- Illumina (MiSeq) and population sequencing performed on genomic length HBV sequences of 157 patients (Micromon, Monash University)
- The average total number of nucleotide reads generated per sample was 312,187 ± 12,327, with an average coverage per site of 14,790 ± 648.
- The threshold for mutation classification was set at a conservative 1%.