

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

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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 ($\geq 45\%$) 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.

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 > 10⁶ 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.

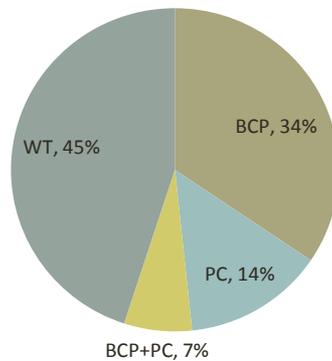
Our primary area of interest was serological response

- 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

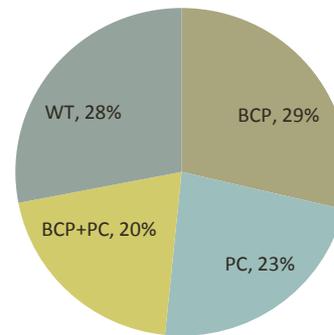
Genotype A	Genotype B	Genotype C	Genotype D	Total
35 (21%)	33 (19%)	54 (32%)	48 (28%)	157

NGS detected BCP and PC mutations with greater sensitivity than Sanger population sequencing

Sanger Population based sequencing
n= 157

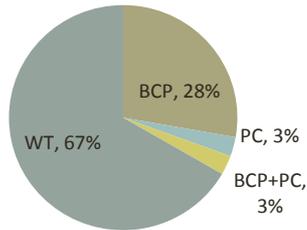


NGS
n=157



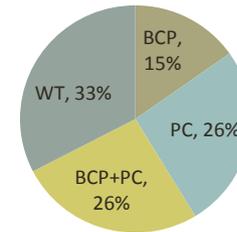
The frequency of BCP and PC mutations varied by genotype

Gt A (NGS, n=36)

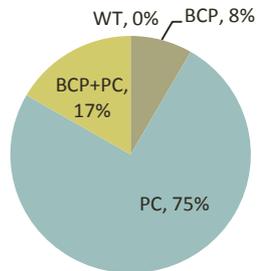


High levels of WT in Gen A and D.

Gt D (NGS, n=46)



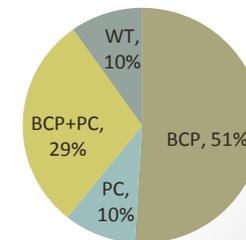
Gt B (NGS, n=24)



Almost no wildtype in Gen B and C!!

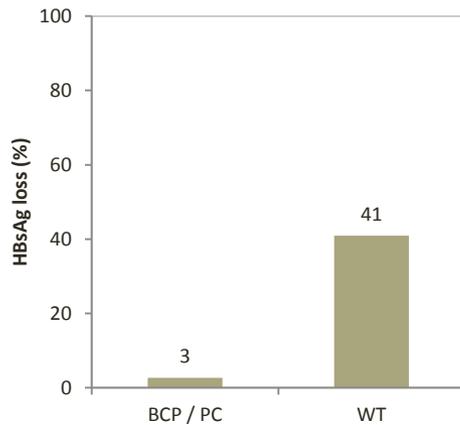
Gen B mostly PC
Gen C mostly BCP

Gt C (NGS, n=51)

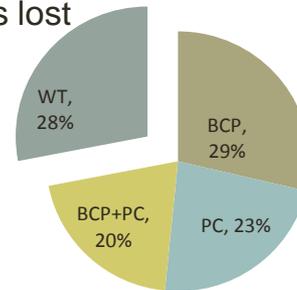


BCP and PC variants were rarely detected in patients who subsequently lost HBsAg

NGS, n = 157



41% of these patients lost HBsAg



Only 3% of patients with these mutations lost HBsAg

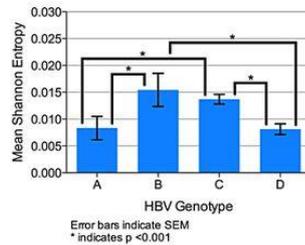
	NPV (BCP/PC)	PPV (WT)
Population seq	99%	21%
NGS	97%	47%

Other Factors

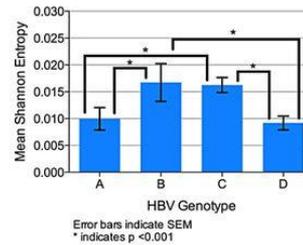
- Duration of infection may be important in likelihood of HBsAg loss.
- Genotypes A and D are almost always acquired by horizontal infection, in adulthood (A) or adolescence (D).
- Genotypes B and C are almost exclusively perinatal transmission, ie birth or very early childhood.
- However, multivariate 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)

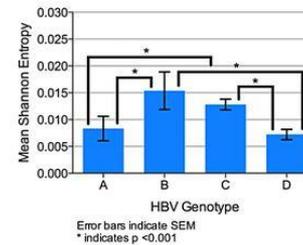
Mean Sequence Diversity by Genotype for Full HBV Genome



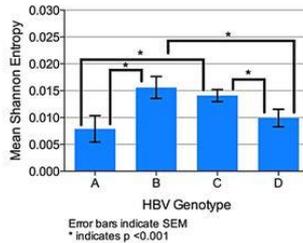
Mean Sequence Diversity by Genotype for Envelope



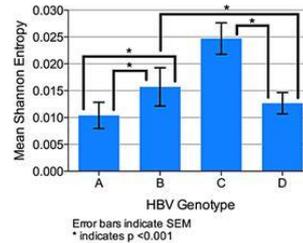
Mean Sequence Diversity by Genotype for Polymerase



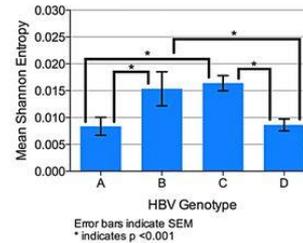
Mean Sequence Diversity by Genotype for Precore/Core



Mean Sequence Diversity by Genotype for NRE-BCP

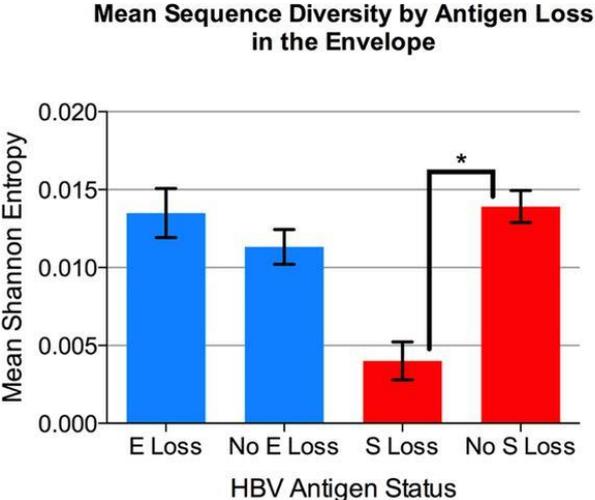


Mean Sequence Diversity by Genotype for HBx

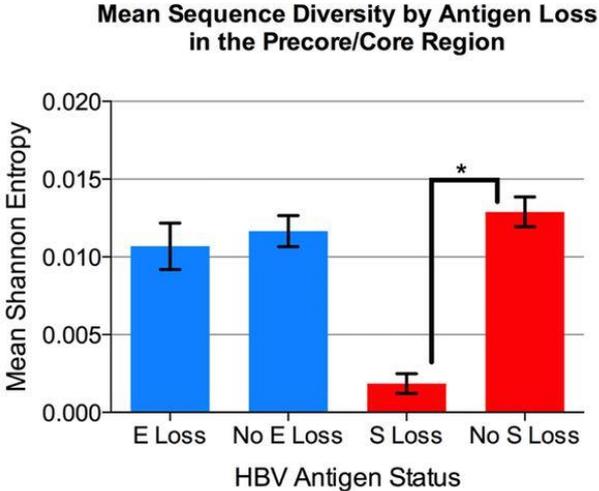


Diversity was always higher in genotypes B/C than A/D.

Increased sequence diversity was associated with reduced likelihood of HBsAg loss (Gen A/D)



Error bars indicate SEM
* indicates $p < 0.001$ on log-transformed data using 2-sample t

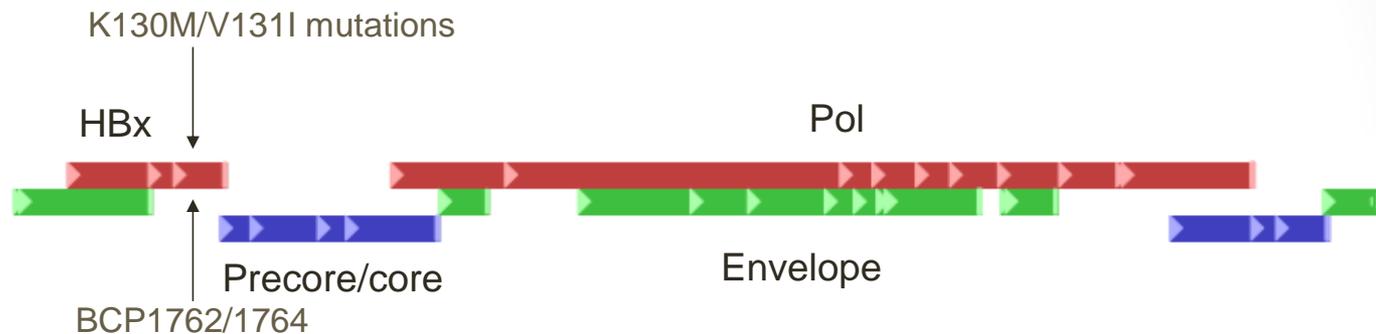


Error bars indicate SEM
* indicates $p < 0.001$ on log-transformed data using 2-sample t

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.

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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 $312,187 \pm 12,327$, with an average coverage per site of $14,790 \pm 648$.
- The threshold for mutation classification was set at a conservative 1%.