Low-Frequency Minority Variants of Hepatitis B Virus Are Associated With Immune Escape Mutations

CROI Conference on Retroviruses and Opportunistic Infections 594

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BACKGROUND

- Hepatitis B virus (HBV) is characterized by extensive genetic diversity, including low-frequency minority variants that contribute to disease progression.
- While these variants represent a critical component of the viral genome pool, their role is often underestimated due to limitations in detection.
- We characterized low-frequency minority variants associated with drug resistance and immune escape mutations and evaluated their potential clinical impact.

METHODS

- We utilized 141 whole-genome sequences generated using next-generation sequencing from Botswana Combination Prevention Project (BCPP) samples to analyze minority variants (<20%).
- BCPP is the largest cohort in Botswana, with over 6,000 individuals screened for HBV to date, and these represent genotypes A1, D3, and E.
- An enhanced in-house bioinformatics suite (HBVgenomer v5.9) was used to analyze for minority variants.
- Minority variants (1-20%) were filtered, and these were compared to previously published drug resistance and escape mutations at the consensus level (>20%).
- The differences in viral diversity and the dominance of escape variants by viral load (VL) categories (Detectable, target not detected (TND)) were compared using the Wilcoxon rank-sum test ($p < 0.05$).

Low-frequency hepatitis B virus variants linked to immune escape are common, and these can behave differently depending on viral load, showing that important viral changes can be missed if only dominant strains are studied.

RESULTS

- Across 83 samples, a total of 516 minority variants were observed, and the **A159T** variant was the most frequent (n = 24).
- Shannon entropy correlated with the top-variant frequency ($p = 0.86$, $p < 0.0001$) in samples with known viral load.
- Including samples without VL slightly reduced the strength of the correlation ($p = 0.76$, $p < 1.9 \times 10°$), yet the relationship remained highly significant (Figure 1).
- When stratified by VL, samples with detectable VL had a median top variant frequency of 4.5% (IQR 3.1-11.2%), compared with 12.5% (IQR 8.2-16.7%) in TND samples (Wilcoxon W = 93, p = 0.004) (Figure 2).

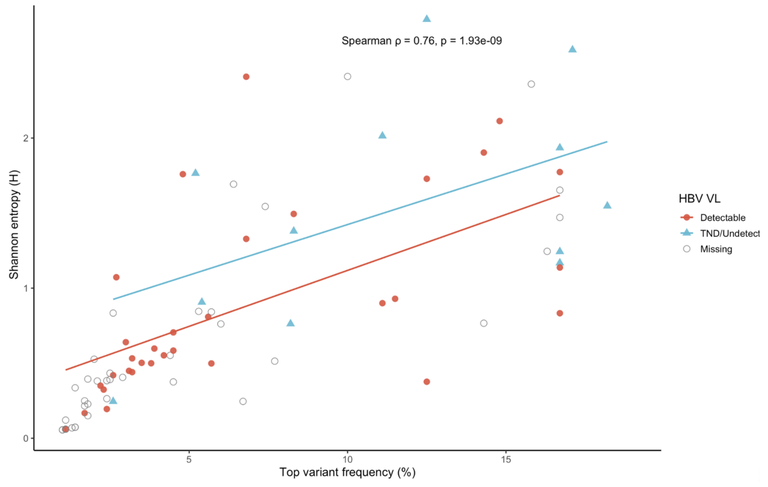


Figure 1 Association of viral diversity with dominant escape variant frequency in HBV

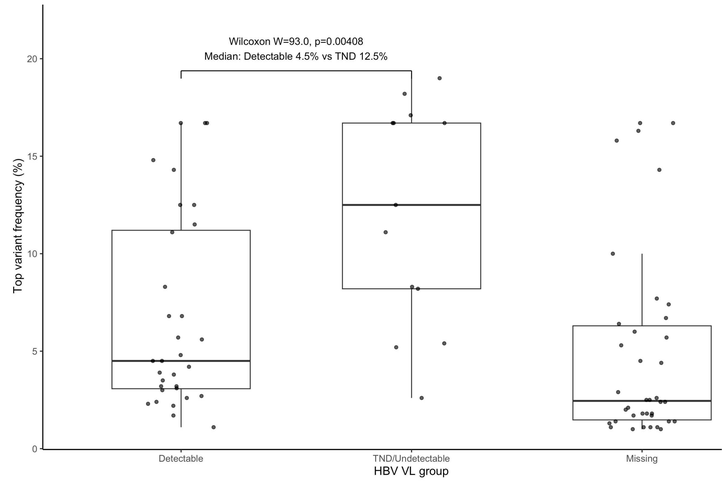


Figure 2 Effect of viral load status on dominant HBV variant frequency

CONCLUSIONS

- We identified minority variants that have been previously suspected to have roles in viral escape, thus highlighting the importance of characterizing minority variants.
- Our findings show that detectable HBV VL is associated with higher diversity, with lower dominance of escape variants (4.5%).
- TNDs who are rarely sequenced in most studies exhibit reduced diversity but stronger dominance of single variants (12.5%).
- Minority escape variants are frequent, and HBV VL influences their distribution and dominance.

ACKNOWLEDGEMENTS

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