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NTCP Polymorphisms Do Not Impact Bulevirtide Antiviral Activity or HDV Treatment Response

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Conclusions

- Clinically relevant NTCP genetic variants are rare and are unlikely to affect interaction with the HDV entry domain
- Bulevirtide demonstrated comparable antiviral activity against HDV across NTCP variants evaluated in vitro.
- The common NTCP synonymous variant G225A was not associated with differences in hepatic NTCP expression.
- Clinical response to Bulevirtide, including HDV RNA decline and ALT normalization, was not impacted by NTCP genetic variation.
- Overall, NTCP polymorphisms are unlikely to influence Bulevirtide efficacy in patients with chronic HDV infection.

Plain Language Summary

- HDV enters liver cells through the NTCP protein, which is blocked by the antiviral drug Bulevirtide.
- Natural genetic differences in NTCP raised the question of whether treatment effectiveness could be affected.
- Laboratory studies showed that Bulevirtide remained highly effective against HDV across all NTCP variants tested.
- In treated patients, NTCP genetic variation did not affect viral decline, liver enzyme improvement, or overall treatment response.

References

1. https://gnomad.broadinstitute.org/
2. Asami J, Park JH, Nomura Y, Kobayashi C, Mifune J, Ishimoto N, et al. Structural basis of hepatitis B virus receptor binding. Nat Struct Mol Biol 2024;31 (3):447-54.
3. Liu H, Zakrzewicz D, Nosol K, Irobalieva RN, Mukherjee S, Bang-Sorensen R, et al. Structure of antiviral drug bulevirtide bound to hepatitis B and D virus receptor protein NTCP. Nat Commun 2024;15 (1):2476.

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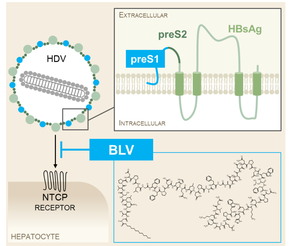
Introduction

- HDV entry into hepatocytes requires the sodium taurocholate cotransporting polypeptide (NTCP).
- Bulevirtide (BLV), a first-in-class entry inhibitor of HDV, is approved in the United States, the European Economic Area, and several other countries for the treatment of patients with chronic hepatitis delta infection with compensated liver disease.
- Naturally occurring NTCP genetic variants are present in the population, but their impact on BLV activity and treatment response is unclear.
- Certain NTCP variants alter susceptibility to HBV infection, raising interest in potential effects on HDV entry inhibition.
- Characterizing NTCP polymorphisms is important for interpreting BLV treatment response and clinical use.

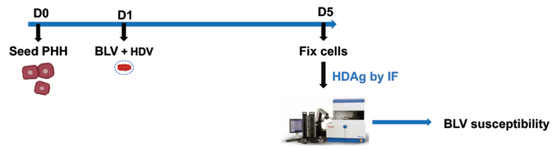
Figure 1. BLV binds to NTCP



Methods

- **Variant selection**: Non-synonymous NTCP variants were selected based on proximity to the PreS1 binding interface in the published NTCP cryo-EM structures and/or minor allele prevalence >0.05% in gnomAD v4.1.
- **Structural analysis**: PreS1–NTCP contact residues were mapped using structural modeling to identify variants within 5 Å of the viral binding domain.
- **In vitro antiviral activity:** Bulevirtide potency was assessed in primary human hepatocytes expressing selected NTCP variants using HDV infection assays.
- **NTCP expression analysis**: Hepatic NTCP mRNA and protein levels were quantified by RNA sequencing and multiplex immunofluorescence in participants from the MYR301 (Phase 3) Bulevirtide study

Figure 2. In Vitro Phenotyping Assay



BLV, bulevirtide; D, day; HDAg, hepatitis delta antigen; HDV, hepatitis delta virus; IF, immunofluorescence; PHH, primary human hepatocyte

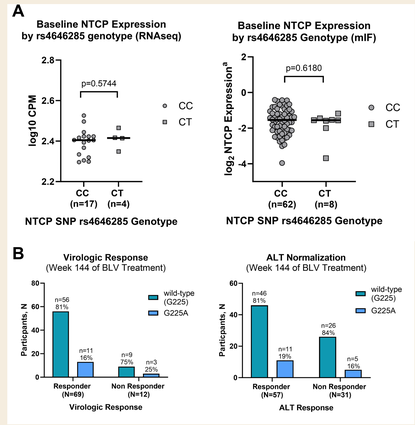
Results

Table 1. NTCP Variants in MYR204 and MYR301 Participants

NTCP SNP	Amino Acid Change	Zygosity	MYR204 (n=143)	MYR301 (n=150)	BLV EC_{50} (nM)
A901G	I301V	Het	1	0	-
C528G	No change	Het	1	0	-
G225A	No change	Hom	26	19	0.64
G573C	No change	Het	1	0	-
G917A, G225A	C306Y, No Change	Het, Het	0	1	-
T503C	I168T	Het	0	1	0.20
T627C	No change	Het	2	1	0.48
T668C	I223T	Hom	1	0	0.11
C800T	S267F	Het	0	0	0.80

Het: heterozygous, Hom: homozygous, SNP: single nucleotide polymorphism

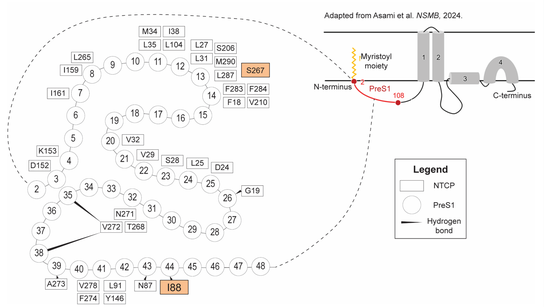
Figure 3. NTCP expression and BLV Clinical Response by rs4646285 GT



[a]log2 NTCP Median Cellular Intensity/Membrane Area
Horizontal lines indicate the median expression per group. P-values between groups were calculated using Mann Whitney U-test.
CPM: counts per million, GT: genotype, mIF: multiplex immunofluorescence, SNP: single nucleotide polymorphism

- BLV retained sub-nanomolar potency against HDV in the presence of NTCP variants identified in BLV clinical studies.
- The most common SNP in MYR301 and MYR204 was rs4646285 synonymous G225A (16%, 46/293).
- No significance was observed between the rs4646285 GT and the NTCP expression, both in the RNAseq and the mIF dataset.
- Virologic response and ALT normalization rates are comparable between synonymous NTCP SNP G225A (rs4646285) status.

Figure 4. PreS1-NTCP Structural Interactions



- Minor allele frequencies of NTCP variants were assessed using gnomAD (v4.1; >1.6M alleles).
- Structural mapping identified 72/349 NTCP residues within 5 Å of PreS1; two of which had MAP >0.05%: S267F (≤0.17%) and I88T (≤0.06%).

Table 2. Summary of NTCP Variants

NTCP Residue	Asami[a]	Liu[b]	5 Å interaction	MYR Studies	Minor Allele Prev (%)[c]	BLV EC_{50} (nM)
64	N	N	N	-	0.01%	-
88	Y	N	Y	-	0.06%	-
131	N	Y	Y	-	0.01%	-
168	N	N	N	I168T (Het)	0.01%	0.2
200	N	N	N	-	0.01%	-
222	N	N	N	-	-	-
223	N	N	N	I223T (Hom)	0.21%	0.11
252	N	N	N	-	0.02%	-
256	N	N	N	-	0.01%	-
267	N	N	Y	-	0.17%	0.8
279	N	N	N	-	0.01%	-
301	N	N	N	I301V (Het)	-	-
306	N	N	N	C306Y (Het)	-	-

[a]PDB: 8HRX, 8HRY (2), [b]PDB: 8RQF (3) [c]gnomAD v4.1 (1)

- BLV retained sub-nanomolar potency against HDV in PHHs expressing S267F, a variant associated with reduced HBV susceptibility.
- I223T was the most frequent variant (≤0.21%) but did not contact PreS1; BLV activity was preserved (EC_{50} = 0.11 nM).