Molecular characterization of HBV and HDV infection in Greenland: Molecular clock analysis of HBV genotype and intrapatient nucleotide diversity during co-infection

Taylor Morriseau¹, Elizabeth Giles¹, Francois Cholette¹, Malene Børresen², Henrik Krarup³, Yasuhito Tanaka⁴, Carla Osiowy¹

1. National Microbiology Laboratory, Winnipeg, Manitoba, Canada
2. Statens Serum Institut, Copenhagen, Denmark
3. Aalborg University Hospital, Aalborg, Denmark
4. Nagoya City University Grad. School of Med. Sci., Nagoya, Japan
1% to 30% depending on region

*7% - 15% HBsAg +

Rex et al 2016 Int J Circumpolar Health 75:29528
Rex et al 2015 World J Hepatol 7:1265
Børresen et al 2011 J Natl Cancer Inst 103:1676
HBV genotypes B and D are highly prevalent throughout the circumpolar Arctic.
HDV antibody prevalence in Greenland

HBV-infected individuals remain at risk of severe disease due to possible superinfection with circulating HDV

68% of HBsAg + were anti-HDV positive
Study Objectives

• What are the circulating HBV/D subgenotypes in Greenland?

• When were these HBV strains introduced to Greenland?

• How does HDV affect the evolution of HBV?
Methods

• 34 samples from 16 individuals granting consent in West Greenland
Methods

• 34 samples from 16 individuals granting consent
  – 8 infected with HBV; 8 infected with HBV-HDV
  – serial, longitudinal samples available from 15 persons (approx. 5 to 10 years apart)

• Sequencing of the full HBV genome (29 samples) or HBsAg coding region only (34 samples)

• Sequencing of HDV (delta antigen and full genome)

• Phylogenetic analysis for tree and distance evaluation

• Evolutionary analysis by Bayesian inference (BEAST 2)

• Estimating the rate of nucleotide substitution by Tree and Rate estimation by Local Evaluation (TREBLE)
HBV Full genome phylogenetic analysis

- □ HBV mono-infected
- ○ HBV-HDV co-infected

*different colours indicate serial specimens from same individual
Differentiation of HBV/D subgenotypes

(def’n of subgenotype: ≥4% nucleotide divergence)

Greenland HBV full genome sequences: nucleotide distance

- D3: 4.0%
- D2: 3.1%
- D1: 2.6%

33-nucleotide deletion at the amino terminus of the preS1 region:
- D: 3,182
- D1: Middle East, Central Asia
- D2: Europe/Japan/Lebanon
- D3: Worldwide
- D4: Australian aborigines, Micronesians, Papua New Guineans, Arctic Denes
- D5: India
- D6: Tunisia/Nigeria
Greenland HBV full genome sequence: analysis of serotype and subgenotype specific substitutions

- Greenland HBV/D sequences are *ayw2*
- Based on specific amino acid substitutions throughout the full genome, neither D1 or D2 are evident (mixture?)

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D2</th>
<th>GL SEQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PreS2 aa39</td>
<td>V/a</td>
<td>A/v</td>
<td>A</td>
</tr>
<tr>
<td>POL Spacer aa100</td>
<td>S/a,t</td>
<td>A/t,v</td>
<td>A</td>
</tr>
<tr>
<td>POL Spacer aa128</td>
<td>S/g</td>
<td>G/s</td>
<td>S</td>
</tr>
<tr>
<td>RT aa126</td>
<td>H</td>
<td>R/h</td>
<td>H</td>
</tr>
</tbody>
</table>

Yousif M, Kramvis A. 2013 Hepatol Res 43:355
HBV/D1 vs. HBV/D2 intergroup divergence
(def’n of subgenotype: ≥4% nucleotide divergence)

Table 1 Mean intragroup and intergroup divergence of the subgenotypes of genotype D determined using 304 sequences downloaded from public databases

<table>
<thead>
<tr>
<th>Subgenotype</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of sequences</td>
<td>153</td>
<td>64</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>D1</td>
<td>2.6 ± 0.5†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>3.1 ± 0.5</td>
<td>2.1 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>3.8 ± 0.6</td>
<td>3.9 ± 0.6</td>
<td>2.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>D4</td>
<td>4.6 ± 0.6</td>
<td>4.5 ± 0.7</td>
<td>4.5 ± 0.7</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td>D5</td>
<td>5.0 ± 0.6</td>
<td>5.0 ± 0.5</td>
<td>4.9 ± 0.6</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>D6</td>
<td>3.7 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>2.6 ± 0.6</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>D7</td>
<td>4.7 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>4.7 ± 0.6</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>D8</td>
<td>5.2 ± 0.5</td>
<td>5.2 ± 0.4</td>
<td>5.2 ± 0.5</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>D8–E‡</td>
<td>5.0 ± 0.4</td>
<td>5.2 ± 0.4</td>
<td>5.4 ± 0.5</td>
<td>4.2 ± 0.4</td>
</tr>
</tbody>
</table>

Yousif M, Kramvis A. 2013 Hepatol Res 43:355
Effect of HDV co-infection on HBV substitution rate (nucleotide distance)

HBV pairwise diversity within groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of seq</th>
<th>Mean %</th>
<th>Std error %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL HBV/HDV</td>
<td>13</td>
<td>0.71%</td>
<td>0.05%</td>
</tr>
<tr>
<td>GL HBV</td>
<td>16</td>
<td>1.48%</td>
<td>0.05%</td>
</tr>
</tbody>
</table>

HBV pairwise diversity between groups

<table>
<thead>
<tr>
<th></th>
<th>GL HBV</th>
<th>SEM %</th>
<th>GL HBV/HDV</th>
<th>SEM %</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>2.78%</td>
<td>0.03%</td>
<td>2.39%</td>
<td>0.03%</td>
</tr>
<tr>
<td>D2</td>
<td>3.23%</td>
<td>0.05%</td>
<td>2.83%</td>
<td>0.05%</td>
</tr>
<tr>
<td>D3</td>
<td>4.04%</td>
<td>0.07%</td>
<td>3.68%</td>
<td>0.09%</td>
</tr>
</tbody>
</table>
Tree and Rate Estimation by Local Evaluation (TREBLE)¹:

Estimates the global rate of nucleotide substitution over time (344 bp HBsAg)

- **HBV-HDV co-infected**
  - n=8
  - Substitutions/site/year: 2.84E-07

- **HBV mono-infected**
  - n=7
  - Substitutions/site/year: 3.10E-05

The Greenlandic HBV/D ancestor emerged ~225 YBP (95% HPD 330-130 YBP)
The HBV/D population has expanded over the last 225-250 years.
Conclusions

• Greenlandic HBV/D clusters with D2 but shares closer nucleotide distance with D1 – mixture or recombinant?

• HDV co-infection contributes to a reduction in the HBV mutation rate – susceptible to immune pressure?

• The HBV/D ancestor appears to have emerged and expanded in Greenland almost 225 years ago
Hepatitis D in Greenland

Full genome sequences; ML analysis (GTR+\gamma)

- GenBank reference sequences
- Greenlandic sequences
HBV genotype D (410 bp HBsAg region) 
(Alaska ●; Greenland ●; D1 ●; D2 ○; D3 ■; D4 □; D5 ▲; D6 △)
HBsAg coding region phylogenetic analysis

- **HBV mono-infected**
- **HBV-HDV co-infected**

*Different colours indicate serial specimens from same individual*