Hepatitis B Virus in African immigrants living in Australia


HBV in Africa

- 240 – 350 million people worldwide living with chronic hepatitis B (CHB)
  - ~65 million reside in Africa

(Ott, Vaccine 2012; Lavanchy, Journal of Viral Hepatitis 2004)

- Predominantly transmitted horizontally during early childhood

African HBV seroprevalence

Hyper-endemic in sub-Saharan Africa


Distribution of HBV genotypes by geographic location on the African continent


HBV in Australia

- In Australia, an estimated 218,000 people (1%) are living with chronic hepatitis B (CHB) the majority (56%) of whom were born overseas
  - Asia/Pacific: 38%
  - Europe: 10%
  - Africa/Middle East: 7%
  - Americas: 0.8%

(MacLachlan, Aust & NZ Journal of Public Health 2013)

- In the past two decades increasing numbers of refugees have come from sub-Saharan Africa (esp. Sudan & Ethiopia)

- People from culturally and linguistically diverse (CALD) backgrounds (particularly Asia-Pacific or Sub-Saharan African background) are listed as a priority population as part of the National Hepatitis B Strategy

Preliminary Studies

- Research project (Elizabeth Bannister, Royal Children’s Hosp, Melb) currently underway to investigate the natural history of CHB in children of African descent living in Australia

- Initial results revealed genotypes A, D and E

- Phylogenetic analysis revealed the presence of subgenotype A1, associated with aggressive clinical disease

- Presence of unusual HBV variants

- Mutational analysis revealed 43% had basal core promoter (BCP) and/or precore (PC) mutations, the majority of whom had seroconverted to anti-HBe
Aim

- To characterise the HBV isolated from adult African immigrants living in Australia (Victoria)
  - Determine the prevalence of the different HBV genotypes/subgenotypes
  - Mutational analysis to determine the presence of clinically significant mutations
    - influence of these factors on clinical outcomes
  - Assist clinicians in the monitoring and treatment of African HBV in Australia

Methods

- 42 African patients (30 males, 12 females) infected with HBV were recruited from 3 clinics in Victoria
  - Royal Melbourne Hosp hepatitis and travel/refugee clinics
  - Geelong Hospital
  - Isis Primary Care (Hoppers Crossing)
- Where possible, demographic, clinical, biochemical and serological data was obtained
- Serum was obtained and HBV DNA extraction performed
- PCR and sequencing of polymerase gene (and overlapping surface gene) and basal core promoter/precore (BCP/PC) gene

Results

- 40 patients had sufficient viral load for polymerase amplification and subsequent genotyping/subgenotyping
- BCP/PC sequencing obtained for 38
- Initial analysis (SeqHepB) revealed
  - 13 patients with genotype A (32.5%)
  - 13 with genotype D (32.5%)
  - 14 with genotype E (35%)

Genotyping

- 10 samples cluster with the A1 subgenotype
- 1 sample each with A4, A5 and A6
- 8 samples cluster with the D7 subgenotype
- 5 samples cluster with the D2 subgenotype

Subtyping of African HBV

Genotype A

- Subgenotype A1
- Subgenotype A4
- Subgenotype A5
- Subgenotype A6

Genotype D

- Subgenotype D2
- Subgenotype D7

Subtyping of African HBV

Genotype A

- MEAGS Maximum Likelihood Tree
  GTReG+I
  (bootstrap 1000x)

Genotype D

- MEAGS Maximum Likelihood Tree
  GTReG+I
  (bootstrap 1000x)
Genotyping of African HBV

Genotype E

14 samples cluster with the E genotype (no subgenotypes)

MEGAS Maximum Likelihood Tree
GTR+G+I (bootstrapped 1000x)

Polymerase and surface gene mutations

- 6 patients currently on AV therapy
  - 5 on entecavir, 1 on tenofovir
  - 1 patient had taken PegIFN for 11 months in 2011
  → none had mutations associated with antiviral resistance

- 1 patient had a rtA181T mutation in the polymerase (associated with resistance to lamivudine, telbivudine and adefovir) in the absence of antiviral therapy

- The same patient had a sP120T mutation in the surface which is associated with HBVg or vaccine escape mutants

Discussion

- Phylogenetic analysis identified a high incidence (25%) of HBV subgenotype A1
  → in young males, A1 is associated with more aggressive clinical disease, with a 4.5 times higher relative risk of developing HCC compared to non-A genotypes (Kramvis and Kew, Hepatology Research 2007)

- Genotype influencing the natural history of CHB in African population (A1 - faster progression)

- High incidence of BCP/PC mutations were observed in all genotypes
  → associated with increased progression to cirrhosis and/or HCC

- These findings have important implications for patient monitoring and treatment, particularly with increasing immigration from the Sub-Saharan region
  → Eg. screen for HCC earlier (fibroscan)

Future studies

- Correlate the African adult and children data
  → seroconversion to anti-HBe occurring early

- Full genome sequencing for more comprehensive mutational and phylogenetic analysis:
  → Eg. PreS1/PreS2, Core, X protein
  → Subgenotyping of 4A-4E (Kramvis, Intervirology 2014; re-classifications)
  → Subgenotyping of D7 (D7/E recombinant; compare with African children)

Frequency of BCP/PC mutations

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<tr>
<th>BCP/PC Changes</th>
<th>Frequency</th>
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<tr>
<td>Mutations identified in 36/38 (95%) PCR +ve patients</td>
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<tr>
<td>1nt insertion/deletion in precore identified in 2 patients</td>
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<tr>
<td>HBe Seroconversion</td>
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<td>Anti-HBe results known for 37 patients</td>
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<td>34/37 patients had seroconverted to anti-HBe</td>
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<td>Others were HBeAg negative</td>
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<td>3 patients were HBeAb-negative, HBeAg positive (BCP mutations only)</td>
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