Hepatitis B Virus in African immigrants living in Australia

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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)

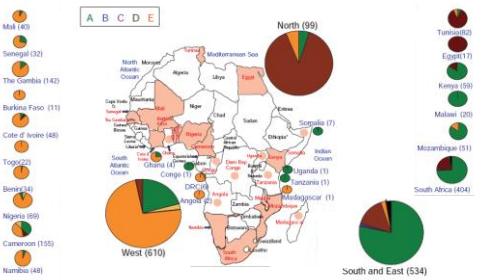
- Predominantly transmitted horizontally during early childhood

African HBV seroprevalence

Hyper-endemic in sub-Saharan Africa

Kramvis and Kew, Hepatology Research 2007, 37: S9-S19

Distribution of HBV genotypes by geographic location on the African continent



Kramvis and Kew, Hepatology Research 2007, 37: S9-S19

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 sero-converted 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

Methods

- HBV consensus sequences were constructed using the DNA sequence analysis program *SeqScape* (Applied Biosystems)
- Consensus sequences were submitted to *SeqHepB* for initial genotyping and a mutational analysis of the Pol/Surface and BCP/PC genes
- For phylogenetic analysis, consensus sequences were compared to a set of published reference sequences (GenBank) representing the 10 human HBV genotypes (A-J, approx 50 subgenotypes)

Results

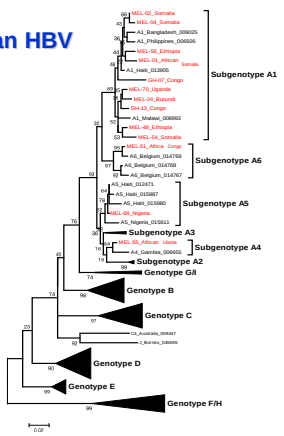
Genotyping

- 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%)

Subtyping of African HBV Genotype A

10 samples cluster with the A1 subgenotype and 1 sample each with A4, A5 and A6

MEGA5
Maximum Likelihood Tree
GTR+G+I
(bootstrapped 1000x)

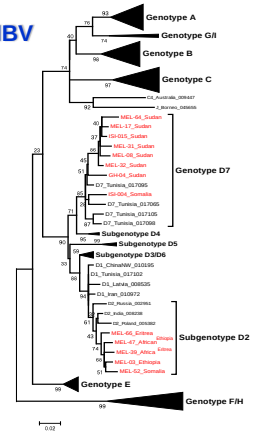


Subtyping of African HBV Genotype D

8 samples cluster with the D7 subgenotype

5 samples cluster with the D2 subgenotype

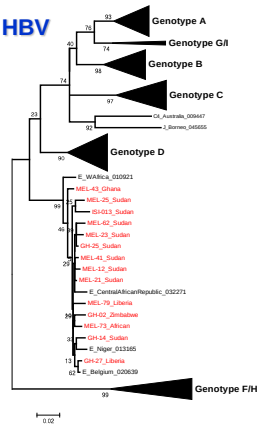
MEGA5
Maximum Likelihood Tree
GTR+G+I
(bootstrapped 1000x)



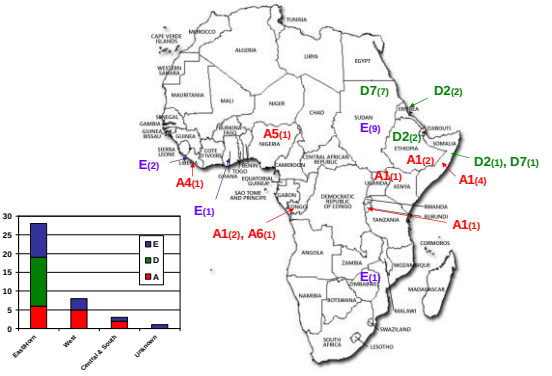
Genotyping of African HBV Genotype E

14 samples cluster with the E genotype (no subgenotypes)

MEGA5
Maximum Likelihood Tree
GTR+G+I
(bootstrapped 1000x)



Genotype/subgenotype distribution by African country of origin



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 HBIg or vaccine escape mutants

Frequency of BCP/PC mutations

BCP	PC	PC Start	A	D	E	Total
A1762T and/or G1764A			5	1	1	7
	G1896A			5	4	9
		M1 I/L/T	3		1	4
A1762T and/or G1764A	G1896A			4	7	11
A1762T and/or G1764A		M1 I/L/T	2			2
	G1896A	M1 I/L/T	1	1		2
A1762T and/or G1764A	G1896A	M1 I/L/T		1		1
Total			11	13	13	36

BCP/PC Changes
- Mutations identified in 36/38 (95%) PCR +ve patients
- 1nt insertion/deletion in precore identified in 2 patients

HBe Seroconversion
- Anti-HBe results known for 37 patients
- 34/37 patients had seroconverted to anti-HBe
- Others were HBeAg-negative
- 3 patients were HBeAb-negative, HBeAg-positive (BCP mutations only)

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 A4-A6 (Kramvis, Intervirology 2014; re-classifications)
  - Subgenotyping of D7 (?D7/E recombinant; compare with African children)

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