

Phylogeography of Hepatitis B virus, sub-genotype C4 in Indigenous Australians

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Introduction

- “Australia antigen” discovered by Blumberg in mid 1960’s from Australian aboriginal serum.
- Sugauchi et al (2001) reported on five full length HBV sequences isolated from Australian aborigines.
 - Two were a novel variant genotype C
 - Three were genotype D (D4)
- **CHARM study**
(Characterising Hepatitis B in Northern Australia through Molecular Epidemiology)

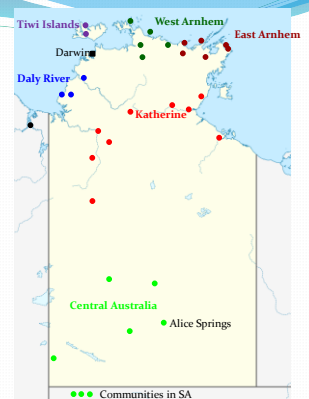
Aim – CHARM study

- Characterise full length, genomic sequences of HBV isolated from chronically infected Indigenous Australians, including documenting the HBV genotypes, sub-genotypes and serotypes circulating in these groups.
- Identify clinically significant mutations that could affect treatment response, vaccine efficacy and treatment failure as well as possible biomarkers associated with liver disease progression.

CHARM study

- 130 HBsAg positive patients recruited to date.
- 39 different communities from 7 regions.
- 100% genotype C4, serotype ayw3
- Full genome HBV sequence from 53 patients.

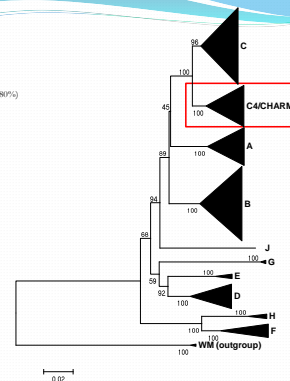
Davies et al. J. Gastro Hepatol 2013;287:1234
Littlejohn et al. J. Med Virol. 2014;86:695



Methods

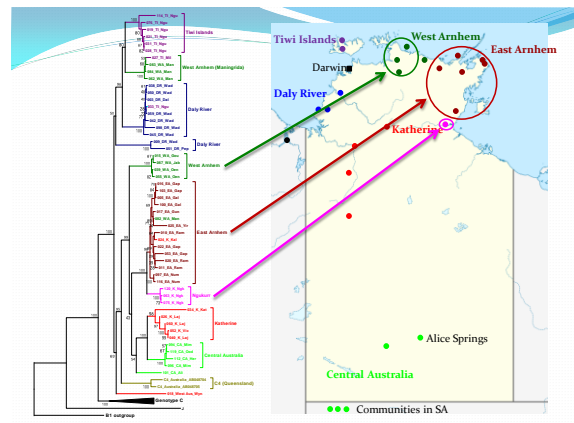
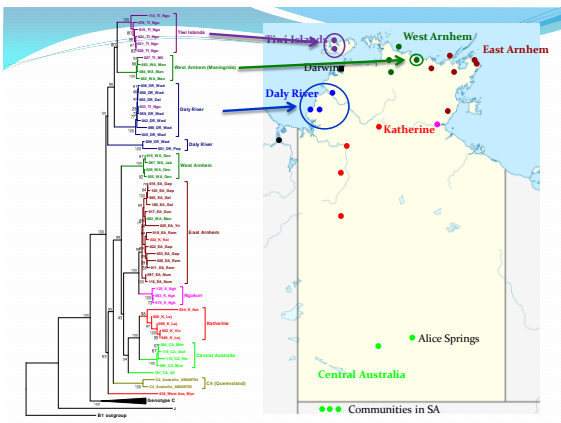
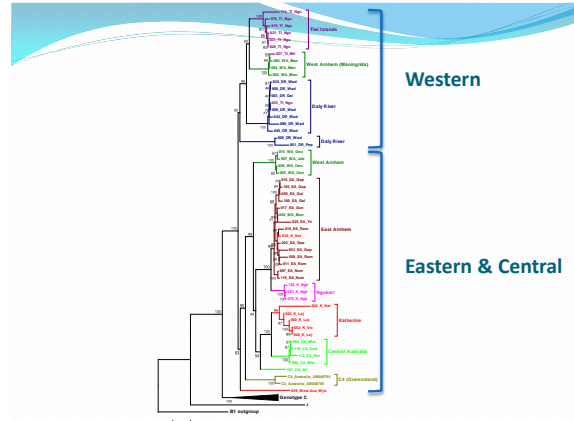
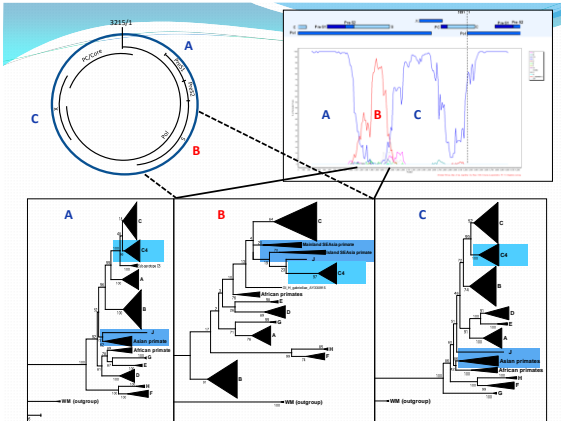
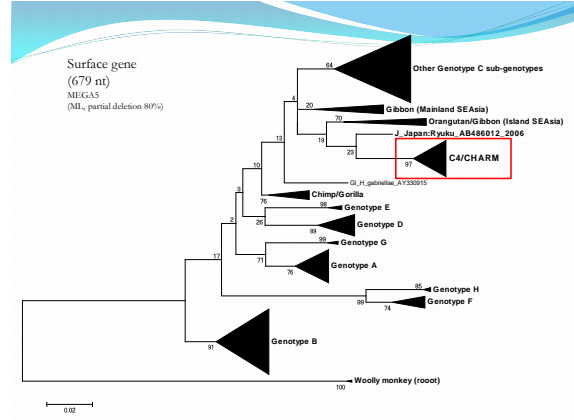
- Serum samples were received at VIDRL, sequencing was carried out on the polymerase/envelope region for initial genotyping.
- Full genome sequence was then obtained from samples with sufficient viral load.
- For phylogenetic analysis, sequences were compared to a set of published reference sequences representing the 10 human HBV genotypes and sub-genotypes.
- Recombination analysis used SimPlot and GroupScan, comparing the sequences to published HBV sequences from all genotypes.
- Mutation analysis was carried out using SeqHepB

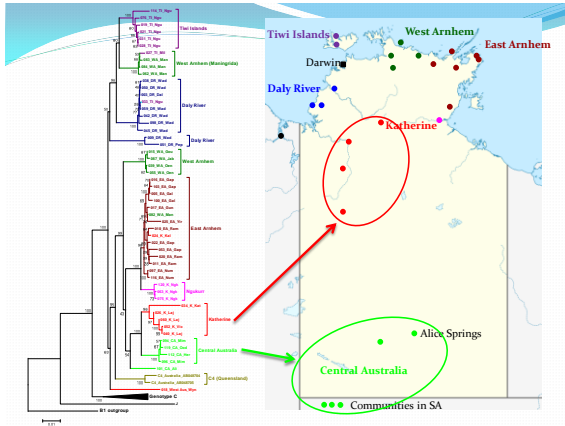
Full Genome
(235 sequences)
MEK4.5
(N), partial deletion 80%



Mean Nucleotide divergence

- Within C4 3.58%
- Between C4 and;
 - Other sub-genotypes of C 6.10 – 7.69%
 - (lowest divergence compared to C3)
 - Other genotypes 9.85 – 15.35%
 - (lowest divergence compared to A)





Conclusions

- The Genotype C4 HBV isolated from indigenous Australians represents a novel strain, serotype ayw3 that appears to be a result of recombination.
- Significantly different from the vaccine strain which is genotype A (adw2). Vaccine failure in this population has been demonstrated.
- Mutation analysis revealed the presence of multiple viral mutations associated with accelerated liver disease progression and an increased risk of HCC.
- Genotype C virus prevalent in indigenous Australians which has important implications for public health as genotype C is the most "oncogenic" of HBV genotypes.
- Phylogenetic analysis shows clustering of the full genome sequences according to geographical region.
- Preliminary evolutionary analysis suggests genotype C4 HBV may have entered Australia with the first Indigenous people.

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Time to MRCA (full genome)

Distance to root node	Subs per site	4.2x10 ⁻⁵ (Fares, 2002)	2.2x10 ⁻⁶ (Paraskevis, 2013)	5x10 ⁻⁷ (Simmonds, 2011)	2-6x10 ⁻⁶ (Gilbert, 2010)
A	0.1055	2,511	47,942	210,946	4,056,660
B	0.1055	2,512	47,958	211,017	4,058,021
C	0.0987	2,350	44,862	197,393	3,796,020
C4	0.0936	2,228	42,526	187,118	3,598,430
D	0.1058	2,520	48,102	211,649	4,070,178
E	0.1083	2,579	49,243	216,668	4,166,696
F/H	0.0914	2,177	41,554	182,836	3,516,071

Phylogeography

