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ORIGINAL ARTICLE

Molecular epidemiology of hepatitis B among Indigenous Australians in Queensland and the Torres Strait Islands

Margaret Littlejohn [1], Lesley-Anne Jaskowski,[2,3] Ros Edwards,[4] Kathy Jackson [4], Lilly Yuen,[4] Darrel Crawford,[2,3] Stephen A. Locarnini[4] and Graham Cooksley[2,3,†]

[1]Victorian Infectious Diseases Reference Laboratory, Royal Melbourne Hospital, and Department of Infectious Disease, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, and [4]Victorian Infectious Diseases Reference Laboratory, Royal Melbourne Hospital, The Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, and [2]Gallipoli Medical Research Institute, Greenslopes Private Hospital, and [3]School of Medicine, University of Queensland, Brisbane, Queensland, Australia

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Correspondence
Margaret Littlejohn, Victorian Infectious Diseases Reference Laboratory, Royal Melbourne Hospital, and Department of Infectious Disease, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Vic., Australia.
Email: margaret.littlejohn@unimelb.edu.au

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Abstract

Background: Chronic hepatitis B virus (HBV) infection is a major health problem for all Indigenous Australians. Post-2000, Hepatitis B surface antigen prevalence has decreased, although remaining four times higher among Indigenous compared with non-Indigenous people.
Aims: This study aimed to characterise the HBV from Indigenous populations in Queensland and the Torres Strait Islands.
Methods: Serum samples were collected, with consent, from people within Queensland Indigenous communities prior to 1990 as part of the Queensland Health vaccination programme. Ethics approval was subsequently obtained to further characterise the HBV from 93 of these stored samples. HBV DNA was extracted and genotype was obtained from 82 samples. HBV full genome sequencing was carried out for a subset of 14 samples.
Results: Seventy-eight samples were identified as genotype C (2 × C12, 3 × C13 and 73 × C14), one sample as genotype A (A2) and three samples as genotype D (1 × D2, 1 × D3 and 1 × D4). The HBV/C sequences identified were most closely related to sequences isolated from Papua New Guinea and Indonesia (Papua Province).
Conclusions: The HBV isolated from the Torres Strait Islanders was notably different to the HBV/C4 strain isolated from Indigenous people of mainland northern Australia, with no evidence of recombination. This reflects the differences in culture and origin between Torres Strait Islanders and mainland Indigenous people.

Introduction

Chronic hepatitis B virus (HBV) infection (CHB) is a major health problem for all Indigenous Australians. Although Hepatitis B surface antigen (HBsAg) has decreased since 2000, it remains four times higher among Indigenous (3.96% (95% confidence interval, CI: 3.15–4.77)) compared to non-Indigenous people (0.90% (95% CI: 0.53–1.28)).[1] Most of the available data examining CHB in Indigenous populations are not region specific. Studies have focused on HBV infection in mainland Indigenous Australians, leading to the identification of the HBV/C4 strain found exclusively in the Northern Territory.[2,3] However, there have been few studies on the prevalence or molecular virology of HBV infection in other regions of Australia, including the Torres Strait Islands (TSI).

The latest data from the Viral Hepatitis Mapping Project: National Report 2017[4] indicate the CHB prevalence in Indigenous people in 'very remote' regions of Queensland, Australia (which includes the Torres Strait) is 4.5%, contrasting with an overall prevalence in Queensland of 0.8% (Indigenous and non-Indigenous), and a national CHB prevalence for Australia of 0.95%. In Far North Queensland (FNQ), treatment uptake was

†Sadly the death of Professor Cooksley during preparation of this manuscript is put on record. The authors dedicate this paper to Professor Cooksley in recognition of his illustrious career and his substantial legacy of work on viral hepatitis in Australia and especially Queensland.
Conflict of interest: None.

2.5% of those with CHB, and care uptake was 20% compared to an overall Australian treatment uptake of 8.3% and similar to the care uptake of 20%.[4] A more recent study of CHB in residents in FNQ found community prevalence varied across the region from 0.4% in western Cape York to 6.8% in the Eastern TSI.[5]

Australian Indigenous people belong to two distinct groups, mainland Indigenous Aboriginals and the TSI people. The Torres Strait encompasses a region between Cape York on the Australian mainland and the island of New Guinea, with numerous islands scattered over 48 000 km^2. The TSI people are culturally and genetically Melanesian, with a relationship closest to Papuans. The population of the TSI is about 8000, of which 82% are Indigenous.[6]

This study aimed to characterise HBV from Indigenous populations in Queensland and the TSI. A previous study was carried out as part of the Queensland Health vaccination programme from 1986 to 1989.[7] From this study, stored samples were available from several Indigenous communities across FNQ. Ethical and community approval was obtained to characterise the HBV from these samples.

Methods

Patient samples and consent

Serum samples had been collected, with consent, from people within Indigenous communities from 1986 and 1989 as part of the Queensland Health vaccination programme.[7] Samples were obtained from 13 different sites across the TSI and mainland Queensland (Fig. 1), with the majority of samples from the Torres Strait communities. Samples were obtained to inform vaccination status for those people who were at risk of HBV infection. This testing programme identified those at risk (who were subsequently vaccinated), those who had anti-HBs (previous exposure) and those who were HBsAg positive (CHB). Ethics approval was obtained for the original study (1986) from the Institute Ethics Committee at Royal Brisbane Hospital.

Ethics approval to further characterise the HBV from 93 stored samples for this current project was obtained from the FNQ Human Research Ethics Committee, and community consent was obtained from four Indigenous community councils, the Torres Strait Island Regional Council, Torres Strait Council, and Kawanyama and Woorabinda communities.

HBV extraction and sequencing

HBV DNA was extracted from 200 μL serum using the QIAamp DNA Minikit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Sequence data for genotyping were obtained for the reverse-transcriptase (RT) region of the HBV polymerase gene (and corresponding overlapping region of the surface (S) gene) (893 bp) as previously described.[2] Population-based HBV full genome sequencing was performed as described previously.[8,9] The full genome sequences have been deposited in the GenBank database (accession numbers MW675885–MW675898).

HBV genotyping

Phylogenetic analysis was carried out to determine the HBV genotype and subgenotype of the isolates. The HBV S gene sequence (681 nucleotides) of the isolates and 311 reference sequences from GenBank representing nine (excluding genotype I) human HBV genotypes were aligned using MAFFT version 7.[10] Maximum likelihood phylogenetic trees were inferred using the maximum composite likelihood G4 model, in MEGA version 5.2.[11] The robustness of the tree structure was assessed with 1000 replicates generated using the bootstrap method.

To confirm the HBV subgenotype of the HBV/C isolates, phylogenetic analysis was carried out using full-length sequences of a subset of isolates and reference sequences from GenBank (140 HBV/C subgenotypes 1–16 and four HBV/B as outgroup). Best-fit substitution model (GTR + F + R5) was determined with ModelFinder[12] based on the Bayesian information criterion. Cluster support was assessed using 1000 pseudo-replicates generated with the UFBoot non-parametric bootstrap procedure[13] implemented in IQ-TREE version 1.6.5.[14]

Genetic distance between HBV/C subgenotypes

The genetic distances between the HBV/C subgenotypes were estimated using the K2 + G (shape = 4) model implemented in MEGA version 5.2.[11] Full-length genome sequences of 152 HBV/C isolates from this study (12 sequences) and GenBank (140 sequences) were used, excluding all nucleotide positions with less than 80% coverage. Standard errors were estimated by bootstrapping (1000 replicates).

Recombination analysis

Recombination analysis was carried out using the SimPlot method.[15] The sequences were tested against consensus sequences generated by SimPlot using the consensus threshold percentage of 50% to the genotype level of 223 full genome HBV sequences from GenBank (39 genotype A, 40 genotype B, 26 genotype C (C4 sequences were not included), 52 genotype D, 10

Figure 1 Map of Queensland and Torres Strait Islands (inset) indicating communities where samples were collected (red dots).

genotype E, 19 genotype F, seven genotype G, 10 genotype H and one putative genotype J), with bootstrapping of 1000 replicates. Window size was 500, with a 20-bp step.

HBV serotyping

HBV serotype was determined by sequencing the HBV S gene. Serotype was deduced from the nucleotide sequence for amino acid positions 122, 127, 134, 140, 159, 160 and 177, according to Purdy *et al.*[16]

Results

Patient samples

A total of 93 samples were consented to be part of this study (Table 1). Samples were predominantly from children (86%) (median age = 7.5 years (range 2–25 years)), and 49% were from females (Table 1). Limited clinical data were available for the samples. The hepatitis B e antigen (HBeAg) status of the individual was known for 85 samples. Of these, a total of 17/85 (20%) were HBeAg negative (median age = 9.5 years (range 3–25 years)) and 68/85 (80%) were HBeAg positive (median age = 6 years (range 2–12 years)) (Table 1).

HBV genotypes, subgenotypes and serotypes detected in initial analysis

Of the 93 samples, HBV genotype was successfully determined for 82 samples. Ten samples were negative for HBV PCR, and sequence data could not be obtained from one sample. Phylogenetic analysis identified 78 genotype C isolates, one genotype A and three genotype D (Fig. 2, Expanded phylogenetic tree in Figure S1). The predominant subgenotypes were HBV/C14 (73/82), followed by HBV/C13 (3/82), HBV/C12 (2/82), HBV/A2 (1/82), HBV/D2 (1/82), HBV/D3 (1/82) and HBV/D4 (1/82) (Table 2). All the HBV/C samples were obtained from TSI communities. The two samples obtained from mainland Queensland sites, Kowanyama (LRC1) and Woorabinda (LRC171), were identified as HBV/A2 and HBV/D3 respectively.

HBV serotyping showed that the HBV/C12 and HBV/C14 isolates were predominantly *adr*q+. However, two of the HBV/C14 isolates were *adw*2, and one other was *adr* indeterminate. The HBV/C13 isolates were *adr* indeterminate. The HBV/D2 isolate was *ayw*3, HBV/D3 and HBV/D4 were *ayw*2, and the HBV/A2 isolate was determined to be *adw*2.

HBV full-length genome analysis

Full-length HBV genome sequencing was performed on a subset of 14 samples to confirm the genotype and subgenotype classifications identified using S gene sequences. This confirmed the subgenotype designations for all sequences, with strong bootstrap support (>70%) (Fig. 3). No recombination events were detected in any of the HBV complete genome sequences. Results of the analysis of the full-length genome sequences for the mean nucleotide divergence are shown in Table 3.

Table 1 Patient demographics for cohort

Community	Total samples	Male	Female	HBeAg positive	HBeAg negative
Torres Strait					
Badu Island	5	0	4	5	0
Boigu Island	7	4	3	5	1
Erub (Darnely) Island	4	3	1	3	0
Kubin (Moa Island)	3	1	0	3	0
Mabuiag Island	5	1	3	4	0
Mer (Murray) Island	4	2	1	2	2
Saibai Island	5	3	2	5	0
St Paul's (Moa Island)	5	1	2	5	0
Ugar (Stephen) Island	3	2	0	2	1
Waiben (Thursday) Island	26	12	11	17	5
Iama (Yam) Island	4	2	1	2	2
Torres Strait (unknown)	20	8	10	14	5
Mainland Queensland					
Kawanyama	1	0	1	0	1
Woorabinda	1	1	0	1	0
	93	40 (51%)	39 (49%)	68 (80%)	17 (20%)

Median age for whole cohort = 7.5 (range 2–25). Data not available for all samples, 79 samples with age data, 85 samples with HBeAg data.

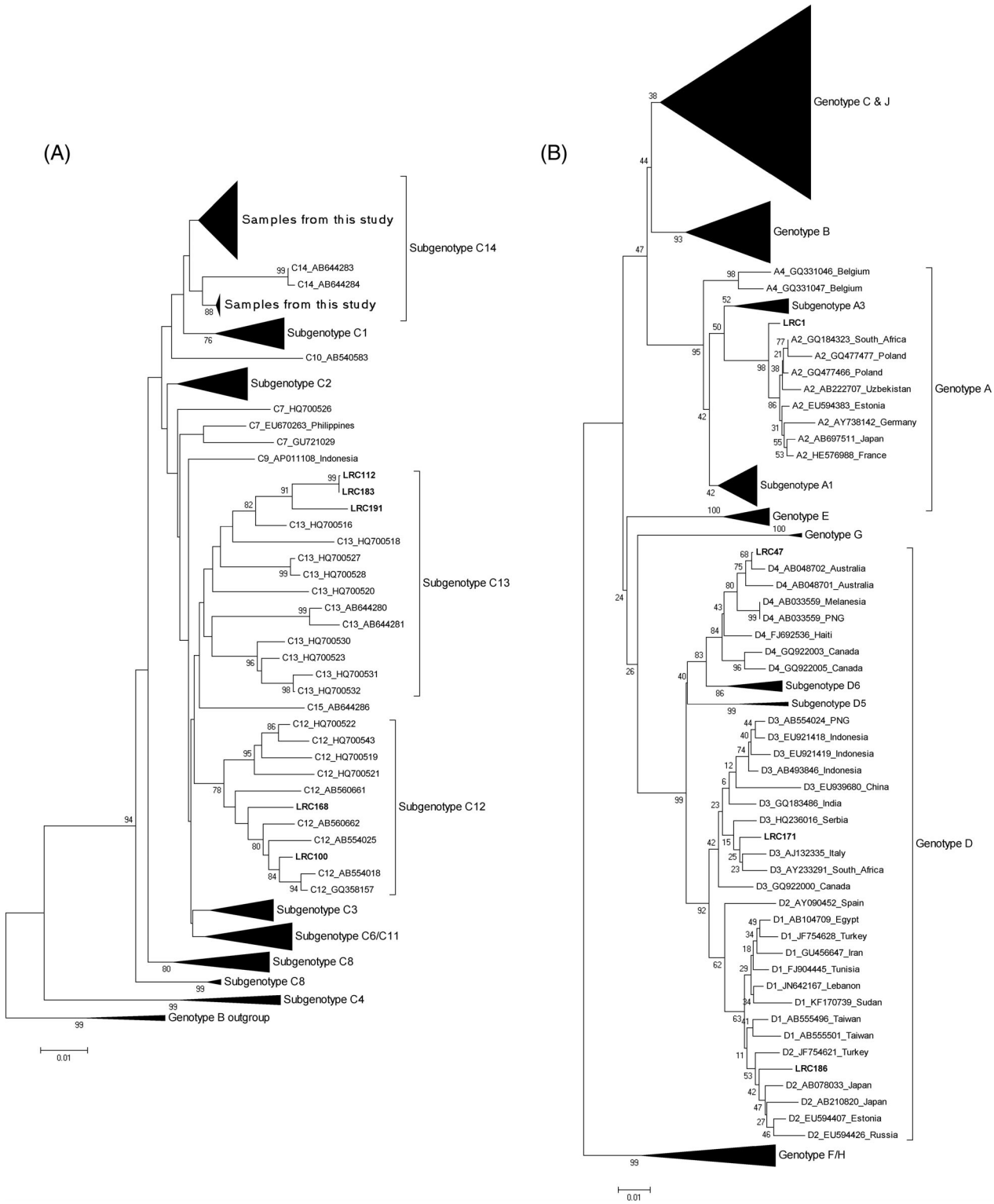

Figure 2 Phylogenetic trees of small surface region (681 nucleotides) constructed using neighbour-joining method. Sequences from GenBank identified by accession number. Sequences from this study are in bold, labelled LRC. (A) All genotype C sequences, with 152 reference sequences from GenBank. (Expanded tree shown in Fig. S1.) (B) All sequences highlighting genotype A and D sequences, with 311 reference sequences from GenBank.

Table 2 Summary of subgenotypes in each community

Subgenotype	Number	Area
A2	1	Kawanyama (mainland Queensland)
C12	2	Thursday Island/Torres Strait
C13	3	Boigu Island/Torres Strait/Waiben Island
C14	73	Torres Strait Islands (Waiben, Badu, St Paul's & Kubin (Moa), Mabuiag, Iama, Saibai, Boigu, Ugar, Erub and Mer)
2	1	Erub Island
D3	1	Woorabinda (mainland Queensland)
D4	1	Waiben Island

For the HBV/C12 subgenotype, the mean nucleotide divergence ranged from 4.76 ± 0.30% with HBV/C16 to 7.04 ± 0.44% with HBV/C10. For the HBV/C13 subgenotype, the mean nucleotide divergence ranged from 4.05 ± 0.25% with HBV/C11 to 6.66 ± 0.40% with HBV/C5 (Table 3).

Characterisation of HBV/C14

The predominant subgenotype detected in samples from the Torres Strait, HBV/C14, was further characterised. Genetic distance analysis of the 12 HBV/C14 sequences (two from Ref. 17, one from Ref. 18 and nine from this study) shows 2.0% nucleotide divergence within the subgenotype. Comparison of these full-length sequences to the reference set of other genotype C subgenotypes showed the closest similarity to HBV/C2 (3.67 ± 0.22% nucleotide divergence) and was most distant to HBV/C4 (6.18 ± 0.38%) (Table 3). Phylogenetic analysis showed that the HBV/C14 sequences formed a monophyletic cluster, separate from the HBV/C2 sequences (Figs. 2, 3).

To identify any molecular features unique to the HBV/C14 subgenotype, the amino acid sequences for the four open reading frames were compared to 140 HBV/C sequences, including all subgenotypes. There were no specific differences noted within the pre-core (PC)/core, X or S open reading frames. Only three residues unique to HBV/C14 were detected, all in the RT and RNaseH region of the polymerase open reading frame (RT_T7S, RT_T128A and RNaseH_S69A). There were several different residues noted compared to the HBV/C4 subgenotype, but for each of these, the HBV/C14 residues were found to match other HBV/C subgenotypes. In addition, there were no notable differences detected at the nucleotide level across the HBV regulatory regions compared to other HBV/C subgenotypes.

Mutation analysis

From the full HBV genome analyses, only one sample had the basal core promoter (BCP) A1762A/T variant associated with reduced HBeAg expression, as a mixed population with wild-type virus. The PC G1896A mutation, which completely abrogates HBeAg expression, was not detected in any sample. The HBV full genome sequences were all obtained from HBeAg-positive patients. None of the nine full genome C14 sequences contained mutations associated with disease progression or of public health significance (BCP_G1613A, C1653T, T1753A/G, Enh1/X promoter G1053A, core deletion or PreS deletion).

Analysis of the 82 HBV genomes for which S gene sequences were obtained showed no encoded antiviral resistance mutations in the overlapping reverse-transcriptase region of the viral polymerase. This was expected as collection dates of the samples pre-dated the era of HBV antiviral therapy. In turn, analysis of the S gene sequence identified no isolates with known vaccine escape variants.

Discussion

In this study, the genotypes and subgenotypes of HBV present in the Indigenous people of Queensland and the TSI were identified. The predominant HBV subgenotype identified in samples from the Torres Strait Islanders was HBV/C14. The HBV isolated from Indigenous people from mainland Queensland was most closely related to European genotypes (HBV/A2 and HBV/D3). It is unknown if the genotype/subgenotype distribution has changed since these samples were collected. Recent studies examining chronic HBV in the FNQ region have not included HBV genotype.[19]

Very few clinically significant variants were identified in this study. This was expected because of the young age of the individuals from which the samples were obtained. The absence of antiviral resistance mutations was expected as the date of collection for these samples pre-dated the availability of antiviral therapy in this region. The proportion of HBeAg-positive samples across this cohort (80%) was consistent with previous studies for this age group,[20] also reflecting the low prevalence of BCP and PC mutations in this cohort. The absence of variants associated with disease progression (BCP and Enh1/X promoter regions) may also be related to the young age of the individuals from which samples were obtained. Further studies in additional age cohorts are required to examine any association between the molecular virology of HBV/C14 and clinical outcomes. The three residues unique to HBV/C14 detected in the

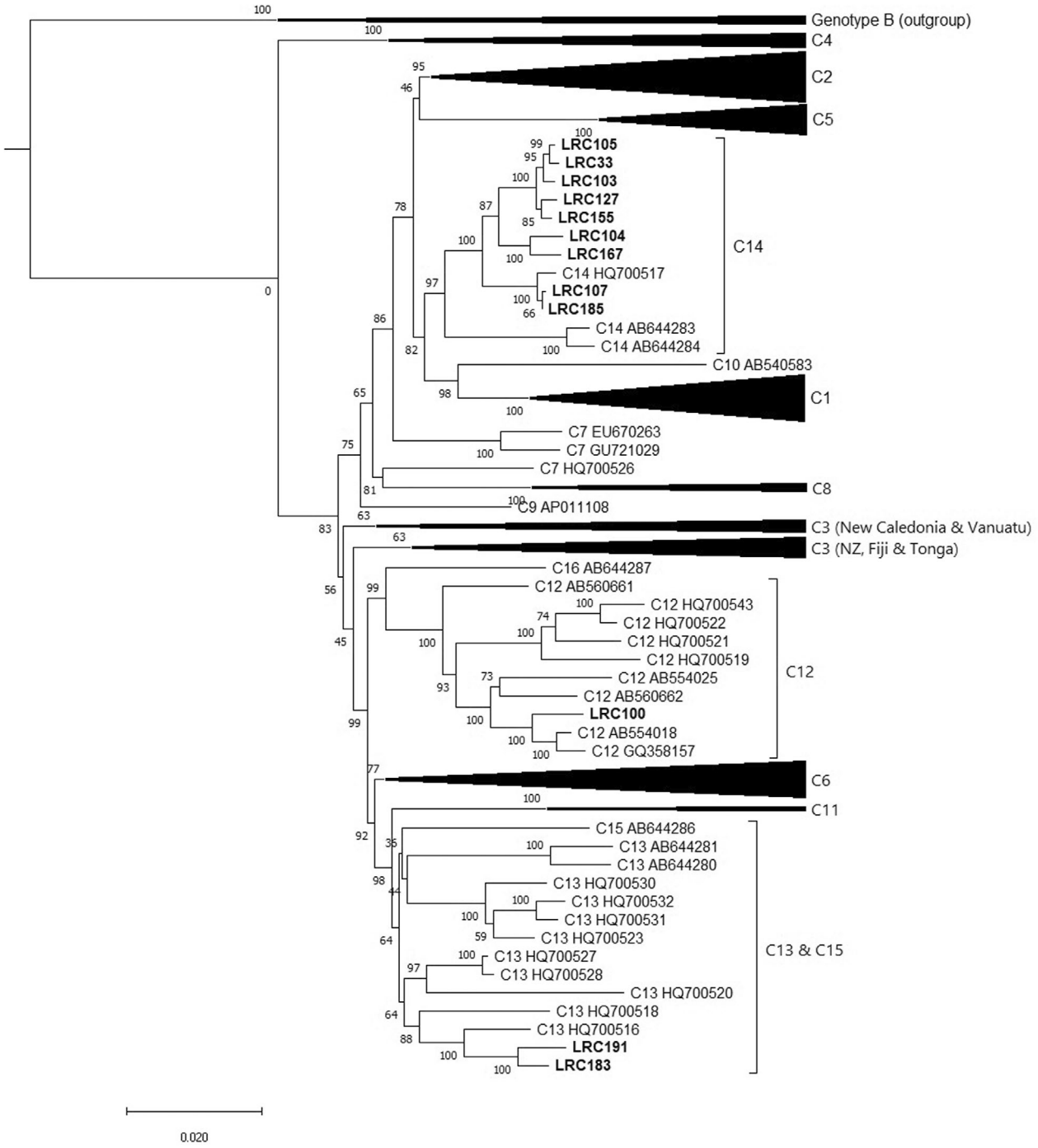

Figure 3 Phylogenetic tree of HBV genotype C full-length genome sequences. Sequences from this study are in bold, labelled LRC. Additional sequences from GenBank are identified by accession number. HBV, hepatitis B virus.

polymerase gene were not situated in any of the conserved active domains; therefore, it is difficult to link these to any potential clinical manifestation.

The genotypes and subgenotypes from most samples from the TSI communities were most closely related to those previously reported from the neighbouring island of New Guinea (both Papua New Guinea and Papua Province, Indonesia). These had been previously identified as subgenotypes HBV/C12, HBV/C13 and HBVC14.[17,18,21] Two isolates from this region were most

Table 3 Genetic distance between HBV/C subgenotypes

Subgenotype	Number of sequences	Divergence HBV/C12	Divergence HBV/C13	Divergence HBV/C14
C1	23	6.50 ± 0.37	5.92 ± 0.33	4.27 ± 0.27
C2	25	5.48 ± 0.30	5.04 ± 0.25	**3.67 ± 0.22**
C3	17	4.91 ± 0.26	4.57 ± 0.23	4.55 ± 0.30
C4	7	6.62 ± 0.37	6.38 ± 0.36	**6.18 ± 0.38**
C5	15	6.91 ± 0.42	**6.66 ± 0.40**	5.43 ± 0.38
C6	17	5.03 ± 0.29	4.31 ± 0.24	4.89 ± 0.32
C7	3	5.29 ± 0.32	5.05 ± 0.30	4.13 ± 0.25
C8	4	5.52 ± 0.33	5.23 ± 0.34	4.59 ± 0.33
C9	1	5.09 ± 0.31	4.6 ± 0.29	4.22 ± 0.32
C10	1	**7.04 ± 0.44**	6.51 ± 0.40	5.15 ± 0.37
C11	2	5.03 ± 0.32	**4.05 ± 0.25**	4.93 ± 0.37
C12	10 (1 this study)		5.03 ± 0.26	5.23 ± 0.34
C13	13 (2 this study)	5.03 ± 0.26		4.86 ± 0.30
C14	12 (9 this study)	5.23 ± 0.34	4.86 ± 0.30	
C15	1	5.25 ± 0.32	4.43 ± 0.28	4.84 ± 0.37
C16	1	**4.76 ± 0.30**	4.57 ± 0.28	4.68 ± 0.35

The number of base substitutions per site from averaging over all sequence pairs between groups is shown (± standard error). Maximum and minimum divergence shown in bold. Total sequences = 152.

closely related to HBV strains detected in Europe (HBV/D2) and some parts of Oceania (HBV/D4).[22]

Surprisingly, HBV/C4, which is the predominant strain found in mainland Australian Aboriginals,[2,3] was not identified in any subjects. Indeed, HBV/C4 was the most divergent genotype C subgenotype compared to the HBV/C14 isolates. This unexpected result is most probably explained by the distinct genetic and cultural divisions between these two populations. Recent analysis of the HBV/C4 subgenotype sequences suggests the HBV/C4 strain entered Australia with the First Australians around 50 000 years ago.[23] This study also suggested the separation of the HBV/C4 and HBV/C3 strains into different populations prior to the entry of anatomically modern humans to Sahul (the combined land mass of Australia and New Guinea). The ancestors of mainland Indigenous Australians entered Australia with the ancestral HBV/C4 strain, whereas the ancestors of Melanesians moved into New Guinea and the Near Pacific with the ancestral HBV/C3 strain. These theories correlate with human genomic data that also suggest the ancestral groups of Indigenous Australians and Papua New Guineans/Melanesians separated at or before entry into Sahul.[24–26] Subsequent to this, it is possible that the ancestral HBV/C3 strain divided into the numerous subgenotypes found today in New Guinea and surrounding regions.

No clear geographical distribution was observed between HBV strains detected among the Torres Strait Islanders, probably reflecting population movement between islands. This is in contrast to the distinct geographical clustering seen in the HBV/C4 strains isolated in Indigenous communities from the mainland Northern Territory region of Australia. The high geographical clustering in this instance possibly results from limited movement between communities.

HBV/C14 was originally described as a novel subgenotype isolated from two samples from the Papua province of Indonesia.[17] This study provides further evidence that HBV/C14 sequences that have only been detected in New Guinea[17,18] and now in the Torres Strait region of Australia form a distinct subgenotype.[22] This was confirmed by (i) phylogenetic analysis of complete genome sequences (12 isolates) that showed HBV/C14 formed a monophyly with strong bootstrap support and (ii) distinct geographical separation between the HBV/C14 and the other HBV/C subgenotypes. HBV/C14 has only been detected in New Guinea and the Torres Strait region of Australia. The most closely related subgenotypes using phylogenetic analysis and nucleotide divergence (C2, C7 and C9) have not been detected in these regions. Nucleotide divergence from HBV/C2 (3.67 ± 0.22%) was slightly below the requisite 4% (accepted divergence for establishing a different subgenotype); however, this criterion is not regarded as binding as the previous two definitions.[22] These results add to the evidence that the HBV/C14 sequences found in the Papua region of Indonesia, Papua New Guinea and the Torres Strait region of Australia form a distinct subgenotype of HBV/C.

The two isolates identified from mainland Queensland communities were European strains. The post-colonial history of Indigenous communities in Queensland involved many instances of resettlement and forced

removal from regions of ancestral origin to missions and reserves.[27] For this reason, it is not surprising that the HBV subgenotypes detected in mainland communities reflected those of colonial European history in the region. The presence of the HBV/D4 subgenotype in the Torres Strait region most probably reflected population movement between the South Pacific Islands and Australia. Subgenotype HBV/D4 has mainly been described from Australia and Micronesia[28]; however, it has also been reported in India[29] and also from Inuit populations in Canada.[30] The pearling industry in the Torres Strait region from the 1880s attracted workers from Japan, Malaya and India as well as forced indentured Pacific Islands (known as 'black-birders'). After this period, there was also forced removal of Pacific Island labourers from the mainland to some TSI. These population movements could explain the presence of this subgenotype.

This study has several limitations. There was little clinical data available associated with the samples, limiting the chance to correlate the HBV subgenotype with clinical outcome. As the samples had been de-identified, no long-term follow-up was possible. In addition, very few samples were available from mainland Queensland.

Conclusion

This study highlights the need for additional studies with Indigenous communities in this region to further characterise both the burden of disease and document the circulating HBV strains. Such studies would enable improved patient management for CHB within the Australian Indigenous communities, where it continues to be a major public health burden.

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Ethics statement

Ethics approval to further characterise the HBV from 93 stored samples for this current project was obtained from the Far North Queensland Human Research Ethics Committee, and community consent was obtained from four Indigenous community councils, the Torres Strait Island Regional Council, Torres Strait Council, and Kawanyama and Woorabinda communities.

Data availability statement

The HBV full genome sequences have been deposited in the GenBank database (accession numbers MW675885–MW675898).

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Figure S1. Expanded phylogenetic tree of the small surface region (681 nucleotides) constructed by the neighbour-joining method of all genotype C sequences, with 152 reference sequences from Genbank identified by accession number. Sequences from this study are in bold, labelled LRC.