Tracing hepatitis B virus (HBV) genotype B5 (formerly B6) evolutionary history in the circumpolar Arctic through phylogeographic modelling

Remco Bouckaert 1, Brenna C. Simons 2, Henrik Krarup 3, T. Max Friesen 4, Carla Osiowy 5, 6

1 Department of Computer Science, University of Auckland, Auckland, New Zealand
2 Alaska Native Tribal Health Consortium, Anchorage, Alaska, United States of America
3 Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark
4 Department of Anthropology, University of Toronto, Toronto, Ontario, Canada
5 National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada
6 Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada

Corresponding Author: Carla Osiowy
Email address: carla.osiwy@phac-aspc.gc.ca

Background. Indigenous populations of the circumpolar Arctic are considered to be endemically infected (>2% prevalence) with hepatitis B virus (HBV), with subgenotype B5 (formerly B6) unique to these populations. The distinctive properties of HBV/B5, including high nucleotide diversity yet no significant liver disease, suggest virus adaptation through long-term host-pathogen association.

Methods. To investigate the origin and evolutionary spread of HBV/B5 into the circumpolar Arctic, fifty-seven partial and full genome sequences from Alaska, Canada and Greenland, having known location and sampling dates spanning 40 years, were phylogeographically investigated by Bayesian analysis (BEAST 2) using a reversible-jump-based substitution model and a clock rate estimated at 4.1x10^{-5} substitutions/site/year.

Results. Following an initial divergence from an Asian viral ancestor approximately 1954 years before present (YBP; 95% highest probability density interval [1188, 2901]), HBV/B5 coalescence occurred almost 1000 years later. Surprisingly, the HBV/B5 ancestor appears to locate first to Greenland in a rapid coastal route progression based on the landscape aware geographic model, with subsequent B5 evolution and spread westward. Bayesian skyline plot analysis demonstrated an HBV/B5 population expansion occurring approximately 400 YBP, coinciding with the disruption of the Neo-Eskimo Thule culture into more heterogeneous and regionally distinct Inuit populations throughout the North American Arctic.

Discussion. HBV/B5 origin and spread appears to occur coincident with the movement of Neo-Eskimo (Inuit) populations within the past 1000 years, further supporting the hypothesis of HBV/host co-expansion, and illustrating the concept of host-pathogen adaptation and balance.
Tracing hepatitis B virus (HBV) genotype B5 (formerly B6) evolutionary history in the circumpolar Arctic through phylogeographic modelling

Remco Bouckaert¹, Brenna C. Simons², Henrik Krarup³, T. Max Friesen⁴, and Carla Osiowy*⁵,⁶

¹Department of Computer Science, University of Auckland, Auckland, New Zealand; ²Alaska Native Tribal Health Consortium, Anchorage, Alaska, USA; ³Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark; ⁴Department of Anthropology, University of Toronto, Toronto, Ontario, Canada; ⁵National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada; ⁶Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada

*Corresponding author:

Name: Carla Osiowy
e-mail: carla.osiowy@phac-aspc.gc.ca
Abstract

Background. Indigenous populations of the circumpolar Arctic are considered to be endemically infected (>2% prevalence) with hepatitis B virus (HBV), with subgenotype B5 (formerly B6) unique to these populations. The distinctive properties of HBV/B5, including high nucleotide diversity yet no significant liver disease, suggest virus adaptation through long-term host-pathogen association.

Methods. To investigate the origin and evolutionary spread of HBV/B5 into the circumpolar Arctic, fifty-seven partial and full genome sequences from Alaska, Canada and Greenland, having known location and sampling dates spanning 40 years, were phylogeographically investigated by Bayesian analysis (BEAST 2) using a reversible-jump-based substitution model and a clock rate estimated at 4.1x10^{-5} substitutions/site/year.

Results. Following an initial divergence from an Asian viral ancestor approximately 1954 years before present (YBP; 95% highest probability density interval [1188, 2901]), HBV/B5 coalescence occurred almost 1000 years later. Surprisingly, the HBV/B5 ancestor appears to locate first to Greenland in a rapid coastal route progression based on the landscape aware geographic model, with subsequent B5 evolution and spread westward. Bayesian skyline plot analysis demonstrated an HBV/B5 population expansion occurring approximately 400 YBP, coinciding with the disruption of the Neo-Eskimo Thule culture into more heterogeneous and regionally distinct Inuit populations throughout the North American Arctic.

Discussion. HBV/B5 origin and spread appears to occur coincident with the movement of Neo-Eskimo (Inuit) populations within the past 1000 years, further supporting the hypothesis of HBV/host co-expansion, and illustrating the concept of host-pathogen adaptation and balance.
**Introduction**

Endemic infection (>2%) with hepatitis B virus (HBV) has been historically observed throughout Inuit and Alaska Native populations of the western circumpolar Arctic (McMahon, 2004; Minuk & Uhanova, 2003; Tulisov et al., 2007), although ongoing vaccination programs, starting in the mid-1980’s to mid-1990’s, have or are expected to reduce prevalence to non-endemic levels (Huynh et al., 2014; McMahon et al., 2011). Studies have shown several HBV genotypes circulating within Inuit or Alaska Native people of the circumpolar Arctic; however, subgenotype B5 (HBV/B5; formerly B6 (Kramvis, 2014)) is unique to this population and has not been found elsewhere (Osiowy, Simons & Rempel, 2013). While chronic HBV infection often results in liver cirrhosis or hepatocellular carcinoma, HBV/B5 chronic infection is infrequently associated with serious adverse effects (Krarup et al., 2008; Minuk et al., 2013; Sakamoto et al., 2007) and rather, results in a ‘benign’ outcome. This clinical association, together with the distinct nucleotide diversity and mutation rate observed with HBV/B5 compared to other HBV genotypes infecting Inuit and Alaska Native populations (Kowalec et al., 2013), suggests potential pathogen attenuation within the population due to host-pathogen co-evolution (Paraskevis et al., 2013; Tedder et al., 2013). There is support for human/HBV coevolution (Paraskevis et al., 2013; Suh et al., 2013), particularly with respect to the distinct geographic distribution of genotypes observed throughout the world and association with specific ethnic groups. In particular HBV genotypes associated with remote or isolated indigenous populations provide a more stable foundation to investigate the coevolving relationship based on the historical HBV endemicity within these populations and their status as the first peoples of a geographic region (Littlejohn, Locarnini & Yuen, 2016; Zehender et al., 2014). In order to investigate the origin, evolution and spread of HBV/B5, spatial and temporal
phylogenetic analysis of HBV sequences obtained throughout the circumpolar Arctic was performed. This analysis should further our understanding of the natural history, origin and evolutionary rate of HBV/B5, which in turn provides increased understanding of HBV evolutionary history and the concept of host-pathogen balance through co-evolution. Through incorporation of viral population size and evolutionary rate estimates, the virus strain could be traced through the circumpolar region to infer the time to the most recent common ancestor (tMRCA) and putative dispersal over time to support long-term association of HBV/B5 with populations indigenous to the western circumpolar region.

Materials and Methods

Serum samples, HBV DNA extraction and sequencing analysis

Twenty-two serum samples from various locations in Nunavut were collected from HBV/B5-infected individuals having self-identified as Inuit. All specimens were described previously in studies for which informed patient consent was obtained for HBV molecular analysis and study approval was granted from institutional ethics review boards (Health Canada/Public Health Agency of Canada research ethics board protocol number REB-2006-0048 and REB-2012-0062; University of Manitoba research ethics board approval number HS15821; (Huynh et al., 2014; Larke et al., 1987; Minuk et al., 2013; Osiowy, Larke & Giles, 2011)). HBV DNA was extracted from 200 μl sera by SDS-proteinase K lysis and phenol chloroform extraction methods (Osiowy, 2002) and resuspended in 30 μl sterile, nuclease-free water. Amplification of the full HBV genome was performed using a high fidelity polymerase (Roche Expand High Fidelity Plus System, Roche Diagnostics, Laval, QC, Canada) as described previously (Osiowy et al., 2010).
Samples having an HBV viral load precluding full genome amplification and sequencing were partially sequenced using primers and methods described previously (Minuk et al., 2012). Specific amplicons were gel-purified prior to cycle sequencing with an AB 3730 XL DNA Analyzer using Big Dye 3.1 terminator chemistry (Thermo Fisher Scientific, Burlington, ON, Canada). Sequences were assembled and analysed using DNA sequence analysis software (Lasergene software suite v 10.0, DNASTAR, Madison, WI, USA). Sequences were submitted to GenBank under accession numbers KP659234-KP659255. Nine other sequences previously described from individuals residing in Nunavut were included (JN792894, JN792896, JN792897, JN792900, JN792901, DQ463795, DQ463796, DQ463799, DQ463802; (Kowalec et al., 2013; Osiowy et al., 2006)). Thus a total of 31 partial and full genome HBV sequences from Nunavut were included in the analysis (Table 1).

Ten serum samples from various locations in Alaska were collected and the HBV DNA extracted and sequenced as described for Nunavut specimens. Participants were enrolled statewide by the Liver Disease and Hepatitis Program at the Alaska Native Tribal Health Consortium (Anchorage, AK). This study was approved by the Alaska Area (Indian Health Service) and the Centers for Disease Control and Prevention Institutional Review Boards (Protocol number AAIRB 2001-07-022/1996-01-001). The study and manuscript were approved by the Alaska Native Tribal Health Consortium and the Southcentral Foundation Board of Directors. All participants provided written informed consent. Sequences were submitted to GenBank under accession numbers KP659219-KP659228. Six other sequences previously described from individuals residing in Alaska were included (AB287314 – AB297319; (Sakamoto et al., 2007)). Thus a total of 16 partial and full genome HBV sequences from Alaska were included in the analysis (Table 1).
Four serum specimens collected from East Greenland were extracted for HBV DNA and sequenced as described for Nunavut specimens. These specimens were collected previously for the study by Krarup, et al. (2008), in which ethics approval was granted by the Commission for Scientific Research in Greenland (Approval number 505-99), and written informed consent was obtained from each participant. Sequences were submitted to GenBank under accession numbers KP659230-KP659233. Six other sequences previously described from individuals located in West Greenland were included (AB287320 – AB287325; (Sakamoto et al., 2007)). Thus a total of 10 partial and full genome HBV sequences from Greenland were included in the analysis (Table 1).

Methods for specimen and data collection and analysis were carried out in accordance with the Tri-Council Policy on Ethical Conduct for Research Involving Humans (Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, and the Social Sciences and Humanities Research Council of Canada).

**Phylogenetic analysis and evolutionary dynamics**

All sequences listed in Table 1, including partial and full genome sequences, were aligned using ClustalX (Thompson et al., 1997), resulting in an alignment with 3220 sites. Bayesian analysis was performed using Markov Chain Monte Carlo (MCMC) methods implemented in BEAST 2 (Bouckaert et al., 2014) for the phylogeny and estimation of the HBV effective population size. In order to facilitate convergence, the MCMC chains were run sufficiently long; at 40 million
generations with sampling every 10000 steps and the first 10% of samples discarded as burn-in.

During analysis, the sequences in the West Greenland clade were restricted to be monophyletic. Without monophyly constraint the analysis did not converge, and from our experience, sequences from the western circumpolar regions of Alaska, Canada and Greenland, tend to cluster into monophyletic clades (Kowalec et al., 2013; Sakamoto et al., 2007). A coalescent tree prior was chosen due to the intra-species analysis and the assumption that sampling across all clades was consistent and accurate. The XML file including all data for BEAST 2 analysis is available as supplemental information (Data S1).

The aligned sequence data consisting of full length and partial sequence was partitioned into eight parts consisting of sites 1-835, 836-1373, 1374-1620, 1621-1900, 1901-2307, 2308-2450, 2451-2847, and 2848-3220, based on gene boundaries in the genome. Since each of these partitions code for different genes, and some even two genes in different reading frames, these partitions will be governed by different evolutionary mechanisms. Therefore, a separate substitution model was used with each partition having its own relative substitution rate. We used the reversible-jump-based substitution model (Bouckaert, Alvarado-Mora & Pinho, 2013) with four gamma categories and invariant sites so that uncertainty in the substitution model choice is integrated out. This model jumps between the models F81 (Felsenstein, 1981), HKY85 (Hasegawa, Kishino & Yano, 1985), TAN93 (Tamura & Nei, 1993), TIM (Posada, 2003), EVS (Drummond & Bouckaert, 2015) and GTR (Tavaré, 1986) and automatically estimates the model parameters during the MCMC.
The phylogenetic tree was prepared using DensiTree implemented in BEAST 2, which has the advantage of being able to visualize the uncertainty in both node heights and topology, such that a qualitative analysis of all tree sets can be made (Heled & Bouckaert, 2013). Bayesian skyline plot analysis was used to estimate the relative HBV/B5 population size through time (Drummond et al., 2005). A coalescent tree prior is used, which is an appropriate prior for within species samples. Furthermore, it uses a non-parametric population function, so there is no commitment to a parametric population function; that is, a population history, such as an exponentially growing population or constant population, is not assumed beforehand. Default settings for the Bayesian skyline plot (five intervals, default hyper priors) were used.

**Geographic analysis**

A joint geographic and phylogenetic analysis of aligned partial and full genome sequences listed in Table 1 using the landscape aware geographic model (Bouckaert et al., 2012) was performed using BEAST 2. Essentially, the model uses a random walk based on successive steps in a random direction to model the spread of organisms across a landscape. The area of interest throughout the western circumpolar Arctic region that is being investigated contains many large landmasses among long stretches of coastline. The landscape aware model was configured to distinguish between sea, inland, and coastal regions and assumed fast (10-fold higher) dispersion rates along the coast compared to dispersion rates inland. Furthermore, there is a reluctance to get into water, but once in water, the rate of dispersal is very high (10-fold higher than along the coast).
A prior on the location of the root of the HBV phylogeographic summary tree was used to enforce its location on the Asian side of the Bering Strait, in order to minimize the geographical area used for the landscape aware model, and to parallel the likely HBV/B5 origin and dispersal from Asia through the Bering Strait region (Paraskevis et al., 2015); thus, the Asian HBV sequences do not impact the geography of the North American and Greenlandic HBV sequences.

During the MCMC run, locations of internal nodes are sampled. To position a branch onto the map, the sample location information is used for the start and end of a branch. To find the location of the mid-point of the branch of length t, the location in a 32x32 grid that maximises the probability of going from a start location to that grid point after time t/2, multiplied by the probability of going from an end location to that grid point after time t/2, is determined. The branch is continually split recursively until all intermediate locations are neighbouring grid points. This determines the most probable path for a branch, which can then be visualised by straight lines connecting grid points. Note that Bayesian analyses do not produce single summary trees (visualised as a blue solid line) but distributions over trees are represented. These are samples from the posterior in the form of a set of trees. Each of these trees follows a somewhat different trajectory and can be visualised individually to get an impression of the uncertainty in the distribution route as well as the area that potentially was visited. Instead of drawing each tree using an opaque line (as was done for the summary tree) a colored dot is drawn using translucency for every grid point in the 32x32 grid traversed by the trajectory associated with the tree. Time depth can be visualised using colour, ranging from light blue (older) to red (younger).

**Results**
HBV/B5 phylogenetic analysis

A total of 72 HBV sequences were included in the phylogenetic analysis; fifty-seven HBV/B5 sequences, of which 40 were full genome sequence, and 11 HBV/B1 and 4 HBV/B2 GenBank-derived full genome sequences used as an outgroup (Table 1). HBV/B1 and B2 sequences were included in the phylogenetic analysis to delineate the ancestral foundation and putative geographic origin of HBV/B5, as HBV/B1 sequences are most phylogenetically similar to HBV/B5 sequences, while HBV/B2 sequences are more distantly related to both B1 and B5 (Sakamoto et al., 2007). Both HBV/B1 and B2 are localized to regions in Asia (Sakamoto et al., 2007) but have not been observed to circulate in Arctic regions. Location information, sample dates and the presence of the precore stop codon mutation (G1896A) for all sequences are listed in Table 1, as are the genomic regions covered for partial sequences. Due to privacy concerns given the small population sizes of the Inuit communities, detailed location information is not given for certain samples not previously described.

Figure 1 shows the relative substitution rates and preferred substitution models for the partitioned data following reversible-jump-based substitution model analysis. Since HBV has a complex genome with many overlapping open reading frames, partitioning can be expected to give a more realistic model of substitution, as each subgenomic region has a different substitution rate (Bouckaert, Alvarado-Mora & Pinho, 2013). The resulting general trends are similar to earlier findings of Bouckaert, Alvarado-Mora & Pinho (2013), such as the requirement for complex substitution models in sequence areas where genes overlap, and higher rates where there is no overlap.
The uncorrelated log-normal relaxed clock (Drummond et al., 2006) fit the data better than the strict clock, as resulting likelihoods and posterior were non-overlapping. Furthermore, the mean coefficient of correlation of 0.63 (95% highest probability density (HPD) Interval [0.52, 0.75]) suggests that the strict clock could be dismissed. The clock was calibrated using archeological evidence from human history, and since humans are the only host for HBV in Arctic regions we can assume they share the same history. Archaeological evidence relevant to a rapid Inuit (Neo-Eskimo) migration to the Eastern Arctic from regions of Alaska starting approximately 900 to 700 YBP (sigma=50 year; (Friesen & Arnold, 2008; McGhee, 2000; Raghavan et al., 2014)) was used to calibrate the root of genotype B coalescence, with an external calibrator estimate of 647 to 953 YBP. This migration displaced earlier Dorset Paleo-Inuit peoples with very little evidence for genetic or cultural interaction between the two populations (Raghavan et al., 2014), thus representing a re-setting of the human population distribution in the Eastern Arctic. Based on the mean external calibrator results, the HBV evolutionary clock rate for this study was estimated at 4.1x10^{-5} substitutions per site per year (95% HPD interval [3.1x10^{-5}, 5.1x10^{-5}]), which is in keeping with median rates from most literature sources, and takes into account the faster rate noted for sequences from HBeAg negative individuals (Harrison et al., 2011), of which most HBV/B5-infected individuals were in the present study due to a high prevalence of the precore stop codon mutation A1896 (Table 1; (Osiowy, Larke & Giles, 2011)). The estimated clock rate gave a root height for genotype B5 of 902 YBP (95% HPD Interval [803-1001]) following DensiTree visualization of the posterior distribution over the set of trees (Fig. 2). This dating is consistent with minimum tMRCA estimates in the literature for genotype B5 (Paraskevis et al., 2015) and corresponds to the external calibrator employed. The DensiTree topology was found
to be well resolved with approximately two thirds of the clades having over 95% posterior

Unexpectedly, the estimated dates derived for the MRCA for HBV/B5 geographic lineages appear to shift in an east to west fashion, such that the tMRCA for the Greenlandic HBV/B5 node is approximately 650 YBP, while the average tMRCA for the Canadian and Alaskan HBV/B5 nodes is approximately 590 YBP. The earlier Greenlandic B5 branch is well supported by an 88.7% probability, while there is uncertainty as to whether subsequent branching is between Nunavut (east and west) and Alaska B5 taxa (47.2% probability) or between east Nunavut and Alaska/west Nunavut B5 (27.1% probability). This demonstrates that the root of HBV/B5 is associated with Greenlandic HBV genomic sequences, thus suggesting phylogenetic evolution of the virus during westward dispersal back into Alaska (Fig. 2).

**HBV/B5 population history and dispersal in the western circumpolar Arctic**

Figure 3 shows the estimated effective number of HBV infections over time, where the y-axis represents the effective population size of HBV. A rapid expansion of the viral population is estimated to have occurred from approximately 400 YBP, which coincides with a rapid diversification shown in Fig. 2 within the same time frame. This coincides with archaeological evidence for a period of transformation in precontact Arctic society, during which a previously relatively homogeneous Thule society transformed into modern Inuit, starting approximately 500 YBP and continuing onwards for several centuries. This transformation is associated with population migrations, environmental shifts, and contact with Europeans (Finkelstein, Ross &
Adams, 2009; Friesen, 2010; McGhee, 1994). An estimate of the current HBV/B5 population size in the order of 100,000 is consistent with current estimated host population size estimates based on population statistics (Central Intelligence Agency, 2015; Statistics Canada, 2014; United States Census Bureau, 2012), in keeping with each infected person hosting an entire HBV quasi-species population (Lauring & Andino, 2010). If the molecular clock rate is increased, this results in reduced population size estimates; thus, confidence in the rate used is provided by the current estimates being consistent with current host populations (Drummond & Bouckaert, 2015). Coincident timing of the HBV population expansion with historical events provides further confidence in the clock rate employed.

The posterior distribution over the HBV/B5 tree sets is shown in Fig. 4 projected onto the map of the western circumpolar Arctic region as a function of increasing time. An animation of the landscape aware geographic model analysis which demonstrates the entire dispersal estimation over time and from which Fig. 4 is taken is provided as a supplemental file (Video S1). The blue line in Fig. 4 represents the path of the maximum clade confidence tree, shown in Fig. 2, and the set of trees representing the posterior is projected onto the map as transparent coloured dots indicating uncertainty in the path of dispersal, especially over northern Nunavut island regions. Thus, the model assumes that HBV/B5 follows a “random walk” through a geographic area, but it does not infer the nature of the host population; i.e., it is neither assumed that the walk occurs through empty space nor populated space, but the virus distribution may approximate the associated (uninfected) host population. The summary tree has good posterior support for most clades (about two third of the clades in the summary tree have over 95% posterior support), except those lower in the tree in Alaska and Nunavut, and there was a clear, monophyletic
separation of geographic HBV sequence clades, with 88.7% and 100% posterior support for the
Alaska/Nunavut and Greenland/Nunavut/Alaska clades, respectively.

The advantage of the landscape aware model is that it allows features of the landscape, such as
water, coastlines and inland areas, to be treated as having different dispersal rates. Original
dispersal of HBV/B5 is estimated to have been more rapid along geographic coastal routes
(Helgason et al., 2006; Maxwell, 1985; Morrison, 1999), similar to what has been described for
HBV genotype C (Littlejohn, Locarnini & Yuen, 2016). The result of the landscape aware
geographic model analysis suggests a rapid evolution of the HBV/B5 ancestor and spread along
geographic coastal routes following its introduction into the Eastern Arctic from Alaska via Asia.
As virus spread along the coast is estimated to be more rapid than inland, modelling tends to
bend long branches along the coast, such as the branch from Asia into Alaska and further
eastward. The modelling reconstruction shows Greenlandic viral evolution transpiring via the
northern islands of Nunavut. The uncertainty of the actual dispersal into Greenland is shown by
the maximum clade confidence tree vs. the posterior distribution (green dots in panel C of Fig. 4)
throughout Ellesmere Island. This uncertainty is likely due to the lack of HBV sequence
coverage in the data set from the more northern regions of Nunavut. However, despite this
limitation, the resulting analysis provides strong statistical support for an early dispersal route to
Greenland through Ellesmere Island. Note the evident walk back towards Alaska in Fig. 4B and
C.

Discussion
Our analysis of HBV/B5 sequences from the western circumpolar Arctic suggests that the virus evolved within Asia approximately 2000 YBP, with a later introduction into the Arctic. The eventual spread of the virus throughout the region possibly coincided with a sudden increase in the effective population of HBV, during the period of Thule Inuit transformation associated with expanded trade interactions and migrations of previously settled populations, creating a population of naïve hosts. Visualisation of phylogeographic analysis suggests that an initial rapid location of the virus to Greenland via Arctic coastal regions was followed by expansion and eventual HBV/B5 spread and evolution in a westward fashion, back to Alaska. A previous study investigating the origins of HBV also used a “coastal trail” model to explain the distribution of HBV genotype C throughout indigenous and relict populations of the Asia-Pacific region (Littlejohn, Locarnini & Yuen, 2016).

Established HBV subgenotypes have been located to East Asia at least 3000 YBP, based on phylogenetic analysis of HBV genotype C2 genomic sequences isolated from a 16th century Korean mummy (Bar-Gal et al., 2012). Furthermore, cladogenesis of the major HBV lineages and genotypes is estimated to have occurred approximately 20,000 YBP, due to major human population migrations (Paraskevis et al., 2013). The tMRCA for genotype B is estimated to have occurred anywhere from approximately 12 to 19.7 thousand YBP, depending on the clock model used (Paraskevis et al., 2013; Paraskevis et al., 2015). The use of external versus internal calibration approaches will influence phylodynamic reconstructions (Zehender et al., 2014). Thus, the use of a slower substitution rate ($5 \times 10^{-5}$ to $<1 \times 10^{-6}$) together with a calibration based on ancient events, such as fossil or human migration data, results in older ancestral nodes, which appears to support the hypothesis of HBV/human host co-expansion (Paraskevis et al., 2015) as a
model of HBV origin. An external or remote calibration approach is appropriate for the present study as genotype B5 is uniquely isolated within western circumpolar Arctic indigenous populations and associations between HBV/B5 molecular and clinical outcome characteristics suggest a long-term history of the virus within this particular population (Kowalec et al., 2013; Sakamoto et al., 2007). As most HBV/B5 is associated with HBeAg negativity (Osiowy, Larke & Giles, 2011), the clock rate is expected to be somewhat faster than is observed with HBeAg positive individuals (Harrison et al., 2011).

The clock rate estimated in the present analysis results in the most recent common ancestor of HBV subgenotypes B1, B2 and B5 to be present approximately 2000 YBP. Assuming this rate is correct; the data demonstrates that B5 intra-evolution and differentiation likely began within HBV-infected populations resident in Asia. Thereafter, the genotype B5 coalescence among the three circumpolar Arctic regions in which HBV/B5 has been observed, occurs approximately 1100 years later (ie. approximately 900 YBP). Surprisingly, the data demonstrates that HBV/B5 from Greenland appears to be the "most ancient", implying that the variant present in modern Greenlandic Inuit diverged from the HBV/B5 ancestor over 650 YBP, while the variants that eventually form Canadian and Alaskan HBV/B5 monophyletic clades started to diverge shortly thereafter. Paraskevis et al., (2013) observed a similar pattern of HBV/B5 viral evolution, such that the HBV/B5 median tMRCA estimate from Alaska was determined to be more recent than that from Canada or Greenland.

Based on archaeological evidence, the eastern Arctic was inhabited by the Paleo-Inuit Late Dorset peoples from approximately 700 to 1300 YBP (Friesen, 2004; Maxwell, 1985). Prior to
this period, the chronology of various cultural groups within the Arctic stems from a migration of earlier Paleo-Inuit peoples from Siberia over 4000 YBP (Achilli et al., 2013; Friesen, 2016; Gilbert et al., 2008). Archaeological evidence further shows that Neo-Eskimo people, also genetically and culturally associated with ancient Siberian peoples (Raghavan et al., 2014), rapidly migrated from regions of Alaska through to the eastern Arctic starting approximately 900 to 700 YBP (Friesen & Arnold, 2008; McGhee, 2000; Moltke et al., 2015; Morrison, 1999). The Earliest Neo-Eskimo migrants to the Eastern Arctic are known as Thule, and are accepted as the ancestors of modern Inuit, with little evidence of genetic mixing between Late Dorset and Thule peoples (Raghavan et al., 2014). By 600-500 YBP, transformation of the homogeneous Thule culture into more diverse cultural groupings had started in a process that lasted for several centuries, based on archaeological observation of shifts in regional populations, settlement patterns and social organization (Friesen, 2010). The impetus for this transformation has been speculated to involve climatic changes leading to increased sea ice and alteration in subsistence sources (Finkelstein, Ross & Adams, 2009) as well as changing internal social networks and increasing external contact and trade with European explorers, whalers and merchants (Friesen, 2010; McGhee, 1994).

The genotype B5 genomic coalescence determined in the present study suggests that HBV/B5 was present in the Neo-Eskimo populations that travelled eastward and populated northwestern Greenlandic sites. Recent evidence points to the pioneering Greenlandic Inuit population as a historically isolated founder population, demonstrated by decreased nucleotide diversity (Moltke et al., 2015). Prolonged isolation among a relatively small, HBV-endemically infected population, may result in viral adaptation, which in turn is associated with a slower viral
evolutionary rate (Lin et al., 2015; Zehender et al., 2014), possibly resulting in slow or static
nucleotide divergence up until the time of HBV population expansion. The Bayesian skyline plot
data shows HBV/B5 expansion between 400 to 250 YBP, coincident with timing of a complex
transformation of the Neo-Eskimo Thule people into more diverse cultural forms consistent with
modern Inuit. Counter-intuitively, the evolutionary history of HBV/B5 within the Arctic appears
to start with a Greenlandic ancestor, by way of an earlier Asian ancestor. However, this pattern
actually fits one of the central aspects of current models of the Thule migration from Alaska to
the eastern Arctic. Archaeological site distributions indicate that the initial migration was rapid,
and involved groups from more than one area in the Alaska / Bering Strait region. The earliest
populations in the northwest Greenland / northern Ellesmere Island region are known as "Ruin
Island Thule", and are widely accepted as; a) among the earliest, or possibly the absolute earliest,
Thule population in the eastern Arctic; b) somewhat different from early Thule elsewhere in the
Canadian Arctic, based on their material culture; and c) originating in the Bering Strait region,
rather than northern Alaska where other migrating Thule populations are likely to have come
from (Friesen, 2016; Gulløv & McGhee, 2006; Marchani, Rogers & O'Rourke, 2007;
McCullough, 1989; Morrison, 1999). The HBV/B5 pattern is consistent with this reconstruction,
and could represent an initial Ruin Island Thule population carrying B5 from the Bering Strait
region into northwest Greenland, followed by migration of other Alaskan Thule populations into
the Canadian Arctic, followed in turn by the spread of B5 back through those later Thule
populations from east to west. This east to west ‘back-migration’ within the high Arctic has been
suggested through tMRCA dating of the Y chromosome (Olofsson et al., 2015), mtDNA variant
analysis (Raff et al., 2015; Tamm et al., 2007), SNP genotyping (Reich et al., 2012) and
linguistic analysis (Hammarström et al., 2015; Raff et al., 2015; Sicoli & Holton, 2014).
Once large scale B5 expansion occurred, horizontal transmission and viral “colonization” likely resulted in a more rapid substitution rate (Lin et al., 2015), eventually leading to an HBV/B5 variant having characteristics observed in modern populations, such as a significantly higher nucleotide diversity (Kowalec et al., 2013; Osiowy et al., 2006) compared to other HBV genotypes which infect indigenous populations, and a high prevalence of the precore A1896 stop codon mutation. HBV/B5 has been hypothesized to function as an “adaptor” variant, such that it excels at escaping host immune selection through its rapid mutation rate, but likely at the cost of high replicative activity, or viral load, thus resulting in lowered transmission. A continued association with the newly infected, yet regionally isolated transitional populations throughout the Arctic would further allow for adaptation in the form of pathogen attenuation, permitting a balance between viral escape leading to persistence, and host immune control, resulting in a lack of immune-mediated liver disease (Minuk et al., 2013).

The present study has several limitations, including sampling bias and a paucity of HBV/B5 sequence data from certain regions. The theory of HBV/B5 evolution and spread in an eastward direction would possibly be supported by obtaining HBV/B5 samples from regions of Siberia to be included in phylogeographical analysis; however, such samples have not yet been identified. HBV co-infection with hepatitis D virus (HDV) has been shown to be high in certain West Greenland communities (Børresen et al., 2010; Langer, Frösner & von Brunn, 1997) and it is possible that co-infection may influence the short-term evolutionary rate of HBV in these individuals, thus affecting the molecular clock rate. Although HBV-infected persons from whom
B5 sequences were obtained from East Greenland were found to be negative for antibody to HDV, the same is not known for the West Greenland sequences.

Conclusions

Through our novel phylogeographic approach, the origin and spread of HBV/B5 throughout the western circumpolar Arctic has been estimated to occur coincident with the movement and settlement of Neo-Eskimo populations within the past 1000 years. Results of this study and the knowledge of the unique association of HBV/B5 with indigenous populations of this region, including historical endemicity and a benign clinical outcome, further support the hypothesis of long-term co-evolution between virus and host, and illustrate the many intricate interactions between a specific variant and an infected population over time.
Acknowledgements

The authors gratefully acknowledge Elizabeth Giles and Chris Huynh for excellent technical assistance in HBV sample sequencing and thank all study participants.
References

Achilli A, Perego U, Lancioni H, Olivieri A, Gandini F, Kashani B, Battaglia V, Grugni V, Angerhofer N, Rogers M, Herrera R, Woodward S, Labuda D, Smith D, Cybulski J, Semino O, Malhi R, Torroni A. 2013. Reconciling migration models to the Americas with the variation of North American native mitogenomes. Proceedings of the National Academy of Sciences USA, 110:14308-14313. DOI:10.1073/pnas.1306290110.

Bar-Gal G, Kim M, Klein A, Shin D, Oh C, Kim J, Kim T, Kim S, Grant P, Pappo O, Spigelman M, Shouval D. 2012. Tracing hepatitis B virus to the 16th century in a Korean mummy. Hepatology, 56:1671-1680. DOI: 10.1002/hep.25852.

Børresen ML, Olsen O, Ladefoged K, McMahon BJ, Hjuler T, Panum I, Simonetti J, Jones C, Krarup H, Koch A. 2010. Hepatitis D outbreak among children in a hepatitis B hyper-endemic settlement in Greenland. Journal of Viral Hepatitis, 17:162-170. DOI: 10.1111/j.1365-2893.2009.01159.x.

Bouckaert R, Alvarado-Mora M, Pinho J. 2013. Evolutionary rates and HBV: issues of rate estimation with Bayesian molecular methods. Antiviral Therapy, 18:497-503. DOI: 10.3851/IMP2656.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C, Xie D, Suchard M, Rambaut A, Drummond A. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. PLoS Computational Biology, 10:e1003537. DOI: 10.1371/journal.pcbi.1003537.
Bouckaert R, Lemey P, Dunn M, Greenhill S, Alekseyenko A, Drummond A, Gray R, Suchard M, Atkinson Q. 2012. Mapping the origins and expansion of the Indo-European language family. *Science*, 337:957-960. DOI: 10.1126/science.1219669.

Central Intelligence Agency. 2015. The World Factbook (Greenland). Available at https://www.cia.gov/library/publications/the-world-factbook/geos/gl.html (accessed 26 October 2016).

Drummond A, Bouckaert R. 2015. *Bayesian Evolutionary Analysis with BEAST*. 1st ed. United Kingdom: Cambridge University Press.

Drummond A, Ho SYW, Phillips M, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLos Biology*, 4:e88. DOI: 10.1371/journal.pbio.0040088.

Drummond A, Rambaut A, Shapiro B, Pybus O. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, 22:1185-1192. DOI: 10.1093/molbev/msi103.

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, 17:368-376.

Finkelstein SA, Ross JM, Adams JK. 2009. Spatiotemporal variability in Arctic climates of the past millennium: Implications for the study of Thule culture on Melville Peninsula, Nunavut. *Arctic, Antarctic, and Alpine Research*, 41:442-454.
Friesen TM. 2016. Pan-Arctic Population Movements: The Early Paleo-Inuit and Thule Inuit Migrations. In: Friesen TM, Mason O, eds. The Oxford Handbook of the Prehistoric Arctic. New York: Oxford University Press, 673-692.

Friesen TM. 2010. Dynamic Inuit social strategies in changing environments: A long-term perspective. Geografisk Tidsskrift-Danish Journal of Geography, 110:215-225.

Friesen TM. 2004. Contemporaneity of Dorset and Thule cultures in the North American Arctic: New radiocarbon dates from Victoria Island, Nunavut. Current Anthropology, 45:685-691. DOI: 10.1086/425635.

Friesen TM, Arnold CD. 2008. The timing of the Thule migration: New dates from the western Canadian Arctic. American Antiquity, 73:527-538.

Gilbert MT, Kivisild T, Grønnow B, Andersen P, Metspalu E, Reidla M, Tamm E, Axelsson E, Götherström A, Campos P, Rasmussen M, Metspalu M, Higham T, Schwenninger J, Nathan R, De Hoog C, Koch A, Møller L, Andreasen C, Meldgaard M, Villems R, Bendixen C, Willerslev E. 2008. Paleo-eskimo mtDNA genome reveals matrilineal discontinuity in Greenland. Science, 320:1787-1789. DOI: 10.1126/science.1159750.

Gulløv HC, McGhee R. 2006. Did Bering Strait people initiate the Thule migration? Alaska Journal of Anthropology, 4:54-63.

Hammarström H, Forkel R, Haspelmath M, Bank S. 2015. Glottolog 2.6 (Jena: Max Planck Institute for the Science of Human History): "Yupik". Available at http://glottolog.org/glottolog?search=Yupik#4/63.03/199.02 (accessed 27 October 2015).
Harrison A, Lemey P, Hurles M, Moyes C, Horn S, Pryor J, Malani J, Supuri M, Masta A, Teriboriki B, Toatu T, Penny D, Rambaut A, Shapiro B. 2011. Genomic analysis of hepatitis B virus reveals antigen state and genotype as sources of evolutionary rate variation. *Viruses*, 3:83-101. DOI: 10.3390/v3020083.

Hasegawa M, Kishino H, Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22:160-174.

Heled J, Bouckaert R. 2013. Looking for trees in the forest: summary tree from posterior samples. *BMC Evolutionary Biology*, 13:221. DOI: 10.1186/1471-2148-13-221.

Helgason A, Pálsson G, Pedersen H, Angulalik E, Gunnarsdóttir E, Yngvadóttir B, Stefánsson K. 2006. mtDNA variation in Inuit populations of Greenland and Canada: Migration history and population structure. *American Journal of Physical Anthropology*, 130:123-134. DOI: 10.1002/ajpa.20313.

Horikita M, Itoh S, Yamamoto K, Shibayama T, Tsuda F, Okamoto H. 1994. Differences in the entire nucleotide sequence between hepatitis B virus genomes from carriers positive for antibody to hepatitis B e antigen with and without active disease. *Journal of Medical Virology*, 44:96-103.

Huynh C, Minuk GY, Uhanova J, Baikie M, Vardy L, Wong T, Osiowy C. 2014. Serological and molecular epidemiological outcomes after two decades of universal infant hepatitis B virus (HBV) vaccination in Nunavut, Canada. *Hepatology*, 60:975A.

Inoue J, Ueno Y, Wakui Y, Fukushima K, Kondo Y, Kakazu E, Ninomiya M, Niitsuma H, Shimosegawa T. 2011. Enhanced replication of hepatitis B virus with frameshift in the precore
region found in fulminant hepatitis patients. *Journal of Infectious Diseases*, 204:1017-1025.

DOI: 10.1093/infdis/jir485.

Koseki T, Hongo S, Muraki Y, Sugawara K, Matsuzaki Y, Nakamura K. 1999. Sequence analysis of the entire genome of hepatitis B virus from a patient with fulminant hepatitis. *Yamagata Medical Journal*, 17:27-40.

Kowalec K, Minuk GY, Børresen M, Koch A, McMahon BJ, Simons B, Osiowy C. 2013. Genetic diversity of hepatitis B virus genotypes B6, D and F among circumpolar indigenous individuals. *Journal of Viral Hepatitis*, 20:122-130. DOI: 10.1111/j.1365-2893.2012.01632.x.

Kramvis A. 2014. Genotypes and genetic variability of hepatitis B virus. *Intervirology*, 57:141-150. DOI: 10.1159/000360947.

Krarup H, Andersen S, Madsen P, Okkels H, Hvingel B, Laurberg P. 2008. Benign course of long-standing hepatitis B virus infection among Greenland Inuit? *Scandinavian Journal of Gastroenterology*, 43:334-343. DOI: 10.1080/00365520701712198.

Langer B, Frösner G, von Brunn A. 1997. Epidemiological study of viral hepatitis types A, B, C, D and E among Inuits in West Greenland. *Journal of Viral Hepatitis*, 4:339-349.

Larke RPB, Froese G, Devine R, Petruk M. 1987. Extension of the epidemiology of hepatitis B in circumpolar regions through a comprehensive serologic study in the Northwest Territories of Canada. *Journal of Medical Virology*, 22:269-276.

Lauring AS, Andino R. 2010. Quasispecies theory and the behavior of RNA viruses. *PLoS Pathogens*, 6:e1001005. DOI: 10.1371/journal.ppat.1001005.
Lin Y, Liu C, Chien W, Wu L, Tao Y, Wu D, Lu X, Hsieh C, Chen P, Wang H, Kao J, Chen DS.

2015. New insights into the evolutionary rate of hepatitis B virus at different biological scales. 
*Journal of Virology*, 89:3512-3522. DOI: 10.1128/JVI.03131-14.

Littlejohn M, Locarnini S, Yuen L. 2016. Origins and evolution of hepatitis B virus and hepatitis D virus. *Cold Spring Harbor Perspectives in Medicine*, 6:a021360.

DOI:10.1101/cshperspect.a021360.

Marchani EE, Rogers AR, O'Rourke DH. 2007. The Thule migration: Rejecting population histories using computer simulation. *American Journal of Physical Anthropology*, 134:281-284.

DOI: 10.1002/ajpa.20650.

Maxwell MS. 1985. *Prehistory of the Eastern Arctic*. Orlando: Academic Press.

McCullough K. 1989. *The Ruin Islanders: Early Thule Culture Pioneers in the Eastern High Arctic*. Ottawa: Canadian Museum of Civilization.

McGhee R. 2000. Radiocarbon dating and the timing of the Thule migration. In: Appelt M, Berglund J, Gulløv HC, eds. *Identities and Cultural Contacts in the Arctic: Proceedings from a Conference at the Danish National Museum, Copenhagen, November 30 to December 2, 1999*. Copenhagen: Danish Polar Center, 181-191.

McGhee R. 1994. Disease and the development of Inuit culture. *Current Anthropology*, 35:565-594.

McMahon BJ. 2004. Viral hepatitis in the Arctic. *International Journal of Circumpolar Health*, 63:41-48. DOI: 10.3402/ijch.v63i0.17784.
McMahon BJ, Bulkow L, Singleton R, Williams J, Snowball M, Homan C, Parkinson A. 2011. Elimination of hepatocellular carcinoma and acute hepatitis B in children 25 years after a hepatitis B newborn and catch-up immunization program. *Hepatology*, 54:801-807. DOI: 10.1002/hep.24442.

Meldal BH, Bon AH, Prati D, Ayob Y, Allain JP. 2011. Diversity of hepatitis B virus infecting Malaysian candidate blood donors is driven by viral and host factors. *Journal of Viral Hepatitis*, 18:91-101. DOI: 10.1111/j.1365-2893.2010.01282.x.

Minuk GY, Kowalec K, Caouette S, Larke B, Osiowy C. 2012. The prevalence and long term outcome of occult hepatitis B virus infections in community based populations. *Journal of Medical Virology*, 84:1369-1375. DOI: 10.1002/jmv.23351.

Minuk GY, Macrury S, Uhanova J, Caouette S, Coleman N, Cummings K, Larke B, Vardy L, Huynh C, Osiowy C. 2013. A paucity of liver disease in Canadian Inuit with chronic hepatitis B virus, subgenotype B6 infection. *Journal of Viral Hepatitis*, 20:890-896. DOI:10.1111/jvh.12121.

Minuk GY, Uhanova J. 2003. Viral hepatitis in the Canadian Inuit and First Nations populations. *Canadian Journal of Gastroenterology*, 17:707-712.

Moltke I, Fumagalli M, Korneliussen TS, Crawford J, Bjerregaard P, Jørgensen M, Grarup N, Gulløv HC, Linneberg A, Pedersen O, Hansen T, Nielsen R, Albrechtsen A. 2015. Uncovering the genetic history of the present-day Greenlandic population. *American Journal of Human Genetics*, 96:54-69. DOI: 10.1016/j.ajhg.2014.11.012.
Morrison D. 1999. The earliest Thule migration. *Canadian Journal of Archaeology*, 22:139-156.

Olofsson J, Pereira V, Børsting C, Morling N. 2015. Peopling of the north circumpolar region - insights from Y chromosome STR and SNP typing of Greenlanders. *PLoS ONE*, 10:e0116573. DOI: 10.1371/journal.pone.0116573.

Osiowy C. 2002. Sensitive detection of HBsAg mutants by a gap ligase chain reaction assay. *Journal of Clinical Microbiology*, 40:2566-2571.

Osiowy C, Giles E, Tanaka Y, Mizokami M, Minuk GY. 2006. Molecular evolution of hepatitis B virus over 25 years. *Journal of Virology*, 80:10307-10314. DOI: 10.1128/JVI.00996-06.

Osiowy C, Kaita K, Solar K, Mendoza K. 2010. Molecular characterization of hepatitis B virus and a 9-year clinical profile in a patient with genotype I. *Journal of Medical Virology*, 82:942-948. DOI: 10.1002/jmv.21758.

Osiowy C, Larke RPB, Giles E. 2011. Distinct geographical and demographic distribution of hepatitis B virus genotypes in the Canadian Arctic as revealed through an extensive molecular epidemiological survey. *Journal of Viral Hepatitis*, 18:e11-e19. DOI: 10.1111/j.1365-2893.2010.01356.x.

Osiowy C, Simons B, Rempel JD. 2013. Distribution of viral hepatitis in indigenous populations of North America and the circumpolar Arctic. *Antiviral Therapy*, 18:467-473. DOI:10.3851/IMP2597.

Paraskevis D, Angelis K, Magiorkinis G, Kostaki E, Ho SYW, Hatzakis A. 2015. Dating the origin of hepatitis B virus reveals higher substitution rate and adaptation on the branch leading to
F/H genotypes. *Molecular Phylogenetics and Evolution*, 93:44-54.

DOI: 10.1016/j.ympev.2015.07.010.

Paraskevis D, Magiorkinis G, Magiorkinis E, Ho SYW, Belshaw R, Allain J, Hatzakis A. 2013. Dating the origin and dispersal of hepatitis B virus infection in humans and primates. *Hepatology*, 57:908-916. DOI: 10.1002/hep.26079.

Posada D. 2003. Using MODELTEST and PAUP* to select a model of nucleotide substitution. *Current Protocols in Bioinformatics*, Chapter 6-Unit 6.5. DOI: 10.1002/0471250953.bi0605s00.

Raff JA, Rzhetskaya M, Tackney J, Hayes MG. 2015. Mitochondrial diversity of Iñupiat people from the Alaskan north slope provides evidence for the origins of the Paleo- and Neo-Eskimo peoples. *American Journal of Physical Anthropology*, 157:603-614. DOI: 10.1002/ajpa.22750.

Raghavan M, DeGiorgio M, Albrechtsen A, Moltke I, Skoglund P, Korneliussen TS, Grønnow B, Appelt M, Gulløv HC, Friesen TM, Fitzhugh W, Malmström H, Rasmussen S, Olsen J, Melchior L, Fuller BT, Fahrni SM, Stafford T,Jr, Grimes V, Renouf MA, Cybulski J, Lynnerup N, Lahr MM, Britton K, Knecht R, Arneborg J, Metspalu M, Cornejo OE, Malaspinas AS, Wang Y, Rasmussen M, Raghavan V, Hansen TV, Khusnutdinova E, Pierre T, Dneprovsky K, Andreasen C, Lange H, Hayes MG, Coltrain J, Spitsyn VA, Götherström A, Orlando L, Kivisild T, Villems R, Crawford MH, Nielsen FC, Dissing J, Heinemeier J, Meldgaard M, Bustamante C, O'Rourke DH, Jakobsson M, Gilbert MT, Nielsen R, Willerslev E. 2014. The genetic prehistory of the New World Arctic. *Science*, 345:1255832. DOI: 10.1126/science.1255832.

Reich D, Patterson N, Campbell D, Tandon A, Mazieres S, Ray N, Parra M, Rojas W, Duque C, Mesa N, Garcia L, Triana O, Blair S, Maestre A, Dib J, Bravi C, Bailliet G, Corach D,
Hunemeier T, Bortolini M, Salzano F, Petzl-Erler M, Acuna-Alonzo V, Aguilar-Salinas C, Canizales-Quinteros S, Tusie-Luna T, Riba L, Rodriguez-Cruz M, Lopez-Alarcon M, Coral-Vazquez R, Canto-Cetina T, Silva-Zolezzi I, Fernandez-Lopez J, Contreras A, Jimenez-Sanchez G, Gomez-Vazquez M, Molina J, Carracedo A, Salas A, Gallo C, Poletti G, Witonsky D, Alkorta-Aranburu G, Sukernik R, Osipova L, Fedorova S, Vasquez R, Villena M, Moreau C, Barrantes R, Pauls D, Excoffier L, Bedoya G, Rothhammer F, Dugoujon J, Larrouy G, Klitz W, Labuda D, Kidd J, Kidd K, Di Rienzo A, Freimer N, Price A, Ruiz-Linares A. 2012. Reconstructing Native American population history. Nature, 488:370-374. DOI: 10.1038/nature11258.

Sakamoto T, Tanaka Y, Simonetti J, Osiowy C, Børresen M, Koch A, Kurbanov F, Sugiyama M, Minuk GY, McMahon BJ, Joh T, Mizokami M. 2007. Classification of hepatitis B virus genotype B into 2 major types based on characterization of a novel subgenotype in Arctic indigenous populations. The Journal of Infectious Diseases, 196:1487-1492. DOI:10.1086/523111.

Sicoli MA, Holton G. 2014. Linguistic phylogenies support back-migration from Beringia to Asia. PLoS ONE, 9:e91722. DOI: 10.1371/journal.pone.0091722.

Statistics Canada. 2014. Aboriginal Peoples in Canada: First Nations People, Métis and Inuit (National Household Survey, Analytical Products, 2011). Available at http://www12.statcan.gc.ca/nhs-enm/2011/as-sa/99-011-x/99-011-x2011001-eng.cfm#wb-head (accessed 26 October 2015).
Sugauchi F, Orito E, Ichida T, Kato H, Sakugawa H, Kakumu S, Ishida T, Chutaputti A, Lai CL, Ueda R, Miyakawa Y, Mizokami M. 2002. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. *Journal of Virology*, 76:5985-5992.

Suh A, Brosius J, Schmitz J, Ole Kriegs J. 2013. The genome of a Mesozoic paleovirus reveals the evolution of hepatitis B viruses. *Nature Communications*, 4:1791. DOI:10.1038/ncomms2798.

Tamm E, Kivisild T, Reidla MM,M., Smith D, Mulligan C, Bravi C, Rickards O, Martinez-Labarga C, Khusnutdinova E, Fedorova S, Golubenko M, Stepanov V, Gubina M, Shadanov S, Ossipova L, Damba L, Voevoda M, Dipieri J, Villes R, Malhi R. 2007. Beringian standstill and spread of Native American founders. *PLoS ONE*, 2:e829. DOI:10.1371/journal.pone.0000829.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10:512-526.

Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences*, 17:57-86.

Tedder RS, Bissett S, Myers R, Ijaz S. 2013. The 'Red Queen' dilemma - running to stay in the same place: reflections on the evolutionary vector of HBV in humans. *Antiviral Therapy*, 18:489-496. DOI: 10.3851/IMP2655.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24:4876-4882.

Tulisov A, McMahon BJ, Koch A, Minuk GY, Chulanov V, Bruce MG, Uhanova J, Børresen M, Williams J, Osiowy C, Gelvan A, Alexeeva M, Larke B, Watt K. 2007. Viral hepatitis in the Arctic. A review from a Circumpolar Workshop on Viral hepatitis, ICCH13. *Alaska Medicine*, 49:193-203.

United States Census Bureau. 2012. American Indian and Alaska Native Population: 2010. Available at [http://www.census.gov/prod/cen2010/briefs/c2010br-10.pdf](http://www.census.gov/prod/cen2010/briefs/c2010br-10.pdf) (accessed 26 October 2015).

Xu Z, Ren X, Liu Y, Li X, Bai S, Zhong Y, Wang L, Mao P, Wang H, Xin S, Wong VW, Chan HL, Zoulim F, Xu D. 2011. Association of hepatitis B virus mutations in basal core promoter and precore regions with severity of liver disease: an investigation of 793 Chinese patients with mild and severe chronic hepatitis B and acute-on-chronic liver failure. *Journal of Gastroenterology*, 46:391-400. DOI: 10.1007/s00535-010-0315-4.

Zehender G, Ebranati E, Gabanelli E, Sorrentino C, Lo Presti A, Tanzi E, Ciccozzi M, Galli M. 2014. Enigmatic origin of hepatitis B virus: an ancient travelling companion or a recent encounter? *World Journal of Gastroenterology*, 20:7622-7634. DOI: 10.3748/wjg.v20.i24.7622.
Figure 1. Relative rates (y-axis) and partitions among the HBV nucleotide site (x-axis), labelled with dominant substitution models for the partition following reversible-jump-based substitution model analysis. The overlapping gene coding regions for the approximately 3200 bp HBV genome are illustrated above the graph (Pol, polymerase). The genome was split into different partitions, since the HBV genome has many regions with overlapping coding frames, and each region can be expected to be governed by different evolutionary processes, explaining the difference in substitution models and average rates. GTR, General Time Reversible Model; TAN, Tamura and Nei; TIM, Transitional Model.
**Figure 2.** DensiTree showing clustering of HBV sequences within geographic locations. The consensus tree is shown by the bold blue line and the estimated tMRCA years before present for tree nodes are shown at the top of the tree. Uncertainty of node heights and topology is shown by the transparent green lines. The clade on the top of the tree ("Asia") consists of HBV genotype B1 and B2 sequences, while all other sequences are HBV/B5. The clade B5 external calibrator estimate was calculated at 647 to 953 YBP based on archeological evidence of the Thule expansion at that time.
Figure 3. Effective population estimates of the HBV/B5 population (y-axis) over the years before present (x-axis) based on the Bayesian skyline plot. The median effective population size is shown by the bold black line, with the 95% highest posterior density indicated in blue. The timeframe spans 1200 YBP to present day.
Figure 4. Estimated HBV dispersal routes into the high eastern Arctic, shown as a progression through time at times 900 YBP (A), 600 YBP (B) and present (C). The blue line indicates the most plausible (highest probability) route of HBV dispersal based on
landscape aware modelling. The coloured dots indicate the posterior distribution of dispersal. The dots are coloured by time, from light blue (earliest), green, yellow, orange, and red (most current). Background map from Wikimedia Commons (https://commons.wikimedia.org/wiki/File:World98.svg). An animation of the landscape aware geographic model analysis which demonstrates the entire dispersal estimation over time and from which Fig. 4 is taken is provided as a supplemental file (Video S1).
### Table 1. Sample date, location, and molecular properties of sequences included in the analysis

| GenBank Accession # | HBV sub-genotype | G1896 A | Full or partial genome | Partial genome region included (nucleotide)* | Location information | Sample dates | Reference |
|---------------------|------------------|---------|------------------------|--------------------------------------------|----------------------|--------------|-----------|
| **ALASKA**          |                  |         |                        |                                            |                      |              |           |
| KP659219            | B5               | A1896   | Full                   |                                            | Alaska              | 07-Feb-2006  | Present study |
| KP659220            | B5               | A1896   | Full                   |                                            | Alaska              | 26-Oct-2005  | Present study |
| KP659221            | B5               | A1896   | Full                   |                                            | Alaska              | 25-Jan-2005  | Present study |
| KP659222            | B5               | A1896   | Partial                | 153-881; 1839-3215                         | Alaska              | 12-May-2003  | Present study |
| KP659223            | B5               | A1896   | Full                   |                                            | Alaska              | 15-Sep-2003  | Present study |
| KP659224            | B5               | A1896   | Full                   |                                            | Alaska              | 10-Dec-2008  | Present study |
| KP659225            | B5               | G1896   | Partial                | 13-866; 1360-1774; 2347-3142               | Alaska              | 01-Sep-2008  | Present study |
| KP659226            | B5               | G1896   | Partial                | 13-884; 1002-1397; 2338-3168               | Alaska              | 18-Jun-2003  | Present study |
| KP659227            | B5               | G1896   | Partial                | 155-834                                    | Alaska              | 14-Dec-2006  | Present study |
| KP659228            | B5               | G1896   | Partial                | 155-834                                    | Alaska              | 29-Apr-2003  | Present study |
| AB287314            | B5               | G1896   | Full                   |                                            | Alaska              | 23-Oct-1973  | Sakamoto et al., 2007 |
| AB287315            | B5               | G1896   | Full                   |                                            | Alaska              | 19-Mar-1975  | Sakamoto et al., 2007 |
| AB287316            | B5               | A1896   | Full                   |                                            | Alaska              | 23-Oct-1973  | Sakamoto et al., 2007 |
| AB287317            | B5               | A1896   | Full                   |                                            | Alaska              | 21-Mar-1974  | Sakamoto et al., 2007 |
| AB287318            | B5               | A1896   | Full                   |                                            | Alaska              | 01-Jan-2004  | Sakamoto et al., 2007 |
| AB287319            | B5               | A1896   | Full                   |                                            | Alaska              | 29-Jan-2004  | Sakamoto et al., 2007 |
| **CANADA**          |                  |         |                        |                                            |                      |              |           |
| KP659234            | B5               | G1896   | Full                   |                                            | West Nunavut        | 08-Oct-1983  | Osiowy, Larke & Giles, 2011; Present study |
| KP659235            | B5               | G1896   | Full                   |                                            | West Nunavut        | 08-Oct-1983  | Osiowy, Larke & Giles, 2011; Present study |
| KP659236            | B5               | A1896   | Partial                | 1-258; 803-1775; 1838-3215                 | West Nunavut        | 21-Apr-1984  | Osiowy, Larke & Giles, 2011; Present study |
| KP659237            | B5               | G1896   | Full                   |                                            | East Nunavut        | 23-Oct-1983  | Osiowy, Larke & Giles, 2011; Present study |
| KP659238            | B5               | A1896   | Partial                | 1-1770; 2375-3215                          | West Nunavut        | 06-Mar-1984  | Osiowy, Larke & Giles, 2011; Present study |
| KP659239            | B5               | A1896   | Partial                | 1-1778; 1839-3215                          | West Nunavut        | 16-Mar-1984  | Osiowy, Larke & Giles, 2011; Present study |
| KP659240            | B5               | A1896   | Partial                | 1-1793; 1838-3215                          | East Nunavut        | 01-May-2012  | Minuk et al., 2013; Present study |
| KP659241            | B5               | A1896   | Partial                | 1-254; 803-1792; 1833-3215                 | East Nunavut        | 28-Apr-2012  | Minuk et al., 2013; Present study |
| KP659242            | B5               | A1896   | Partial                | 1-253; 780-1770; 1840-2848; 2867-3215      | East Nunavut        | 27-Apr-2012  | Minuk et al., 2013; Present study |
| KP659243            | B5               | A1896   | Partial                | 1-898; 969-1333; 1666-2405; 2825-3215       | East Nunavut        | 15-Sep-1983  | Osiowy, Larke & Giles, 2011; Present study |
| KP659244            | B5               | A1896   | Partial                | 1-1792; 1839-3215                          | East Nunavut        | 26-Apr-1983  | Osiowy, Larke & Giles, 2011; Present study |
| KP659245            | B5               | A1896   | Full                   |                                            | East Nunavut        | 09-May-2012  | Minuk et al., 2013; Present study |
| KP659246            | B5               | A1896   | Full                   |                                            | East Nunavut        | 23-Oct-1983  | Osiowy, Larke & Giles, 2011; |
| Accession | Type | Length | Region | Date        | Ref.                                      |
|-----------|------|--------|--------|-------------|-------------------------------------------|
| KP659247  | B5   | G1896  | Full   | East Nunavut | 05-May-1983, Osiowy, Larke & Giles, 2011; Present study |
| KP659248  | B5   | A1896  | Full   | East Nunavut | 29-Apr-2013, Huynh et al., 2014; Present study |
| KP659249  | B5   | A1896  | Full   | East Nunavut | 01-May-2013, Huynh et al., 2014; Present study |
| KP659250  | B5   | A1896  | Full   | East Nunavut | 17-Jun-2013, Huynh et al., 2014; Present study |
| KP659251  | B5   | A1896  | Full   | East Nunavut | 10-Apr-2013, Huynh et al., 2014; Present study |
| KP659252  | B5   | A1896  | Full   | East Nunavut | 22-May-2013, Huynh et al., 2014; Present study |
| KP659253  | B5   | A1896  | Full   | East Nunavut | 22-May-2013, Huynh et al., 2014; Present study |
| KP659254  | B5   | A1896  | Full   | East Nunavut | 27-May-2013, Huynh et al., 2014; Present study |
| KP659255  | B5   | A1896  | Full   | East Nunavut | 17-Jun-2013, Huynh et al., 2014; Present study |
| JN792894  | B5   | A1896  | Full   | East Greenland | 01-Sep-2009, Kowalec et al., 2013 |
| JN792896  | B5   | A1896  | Full   | East Greenland | 01-Sep-2009, Kowalec et al., 2013 |
| JN792897  | B5   | A1896  | Full   | East Greenland | 01-Sep-2009, Kowalec et al., 2013 |
| JN792900  | B5   | A1896  | Full   | East Greenland | 01-Sep-2009, Kowalec et al., 2013 |
| JN792901  | B5   | A1896  | Full   | East Greenland | 01-Sep-2009, Kowalec et al., 2013 |
| DQ463795  | B5   | A1896  | Full   | West Greenland | 05-Apr-2004, Osiowy et al., 2006 |
| DQ463796  | B5   | A1896  | Full   | West Greenland | 05-Apr-2004, Osiowy et al., 2006 |
| DQ463799  | B5   | A1896  | Full   | West Greenland | 05-Apr-2004, Osiowy et al., 2006 |
| DQ463802  | B5   | A1896  | Full   | West Greenland | 05-Apr-2004, Osiowy et al., 2006 |
| **GREENLAND** | | | | | |
| KP659230  | B5   | A1896  | Partial | East Greenland | 01-Nov-1998, Present study |
| KP659231  | B5   | A1896  | Partial | East Greenland | 01-Nov-1998, Present study |
| KP659232  | B5   | A1896  | Partial | East Greenland | 01-Nov-1998, Present study |
| KP659233  | B5   | A1896  | Partial | West Greenland | 01-Nov-1998, Present study |
| AB287320  | B5   | A1896  | Full   | West Greenland | 01-Jan-1998, Sakamoto et al., 2007 |
| AB287321  | B5   | A1896  | Full   | West Greenland | 01-Jan-1998, Sakamoto et al., 2007 |
| AB287322  | B5   | A1896  | Full   | West Greenland | 01-Aug-2004, Sakamoto et al., 2007 |
| AB287323  | B5   | A1896  | Full   | West Greenland | 01-Jan-1998, Sakamoto et al., 2007 |
| AB287324  | B5   | A1896  | Full   | West Greenland | 01-Aug-2004, Sakamoto et al., 2007 |
| AB287325  | B5   | A1896  | Full   | West Greenland | 01-Aug-2004, Sakamoto et al., 2007 |
| **ASIA** | | | | | |
| AB010289  | B1   | G1896  | Full   | Japan | 01-Jan-1993, Koseki et al., 1999 |
| AB010290  | B1   | A1896  | Full   | Japan | 01-Jan-1993, Koseki et al., 1999 |
| AB010291  | B1   | A1896  | Full   | Japan | 01-Jan-1993, Koseki et al., 1999 |
| AB010292  | B1   | A1896  | Full   | Japan | 01-Jan-1993, Koseki et al., 1999 |
| Accession  | Location  | Length | Date     | Reference               |
|------------|-----------|--------|----------|-------------------------|
| AB073838   | B1        | A1896  | Japan    | 01-Jan-2001             | Sugauchi et al., 2002 |
| D23677     | B1        | G1896  | Japan    | 01-Jan-2000             | Horikita et al., 1994 |
| D23678     | B1        | A1896  | Japan    | 01-Jan-2000             | Horikita et al., 1994 |
| D23679     | B1        | A1896  | Japan    | 01-Jan-2000             | Horikita et al., 1994 |
| AB287326   | B1        | A1896  | Japan    | 01-Jan-2006             | Sakamoto et al., 2007 |
| AB287327   | B1        | A1896  | Japan    | 01-Jan-2006             | Sakamoto et al., 2007 |
| AB602818   | B1        | G1896  | Japan    | 01-Aug-2005             | Inoue et al., 2011    |
| FJ386584   | B2        | G1896  | China    | 21-Feb-2008             | Xu et al., 2011       |
| FJ386600   | B2        | G1896  | China    | 03-Mar-2008             | Xu et al., 2011       |
| FJ386636   | B2        | G1896  | China    | 22-Feb-2008             | Xu et al., 2011       |
| GQ924653   | B2        | G1896  | Malaysia | 27-Jan-2007             | Meldal et al, 2011    |

* Nucleotide numbering based on GenBank accession no. DQ463795 (3215nt in length)