

Molecular characteristics and clinical relevance of African genotypes and subgenotypes of hepatitis B virus

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Hepatitis B virus (HBV), a DNA virus, replicates via an RNA intermediate, through reverse transcription catalysed by the viral polymerase that lacks proof reading ability. Thus sequence heterogeneity is a feature of HBV being classified into at least 9 genotypes and over 35 subgenotypes. Africa has a high diversity of genotypes/subgenotypes, with distinct geographical distributions. Genotype A is found mainly in south-eastern Africa, E in western and central Africa and D prevailing in northern Africa. Outside Africa, subgenotype A2 prevails and A1 in Africa, which was the most probable source of its dispersal to Asia and Latin America, as a result of slave and trade routes. Genotype E is also an African strain with low genetic diversity, intimating a recent emergence of 200 years or less, with its dispersal outside Africa occurring as a result of modern human migrations. Carriers of subgenotype A1 and genotype E display unique clinical features. A1-infected individuals have low viral loads, low frequency of HBeAg-positivity, horizontal transmission of HBV, higher levels of liver damage and a higher risk of developing hepatocellular carcinoma. In contrast, individuals infected with genotype E have high viral loads, high frequency of HBeAg-positivity and transmit HBV perinatally. Although 15% of HBV infections in HIV-infected Africans are HBsAg-negative, the true occult phenotype of low viral loads is found in only 7% and 65% of individuals infected with subgenotype A1 and genotypes E (or D), respectively. Molecular and functional characteristics of these African HBV strains can account for their different clinical manifestations.

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Africa is the only continent where chronic hepatitis B virus (HBV) infection remains hyperendemic with exposure, as measured by anti-HBc-positivity, of greater than 85% in western Africa, 65 - 85% in eastern Africa and 35 - 75% in southern Africa.^[1] A quarter of the world's 257 million chronic carriers of HBV reside in Africa and this figure most probably underestimates the true carrier rate due to underreporting and a lack of extensive surveillance. As a consequence of the high chronic HBV carrier rate there is a correspondingly high annual incidence of hepatocellular carcinoma (HCC) of 14.8 per 100 000 in males and 6.2 per 100 000 in females,^[2] ranking second and fifth of the leading cancer-associated deaths for men and women, respectively.^[3] In western Africa, HCC is the most commonly diagnosed cancer in males, with the highest annual fatality ratio for any human tumour of 0.93.^[4] Chronic HBV infection is the primary aetiological risk factor for the development of HCC in Africans, with two-thirds of children and up to 80% of adults being carriers of the hepatitis B surface antigen (HBsAg).^[5,6] Apart from chronic HBV infection, other co-carcinogens contributing to the development of HCC in Africa include dietary iron overload^[7] and aflatoxin B1.^[8]

Genotypes and subgenotypes of HBV

Although HBV has a DNA genome, it replicates via an RNA intermediate, through the process of reverse transcription catalysed by a viral polymerase that lacks proofreading ability. Sequence heterogeneity is therefore a feature of the virus. Currently, at least 9 genotypes and over 35 subgenotypes of HBV are recognised globally^[9] with distinct geographical distributions. The genotypes prevalent in south-east Asia differ from those in Africa.^[9,10]

Geographical distribution of HBV genotypes in Africa

Africa has a high diversity of genotypes and subgenotypes displaying distinct geographical distributions. Genotype A is found mainly

in south-eastern Africa, genotype E in western and central Africa and genotype D prevails in northern Africa.^[1] We have identified a unique segment of genotype A, known as subgenotype A1,^[11,12] which is the prevalent subgenotype of A found in Africa. Subgenotype A2 predominates outside Africa^[13] and genotype E, a predominantly African strain, is endemic in western and central Africa and has low genetic diversity reflecting emergence less than 200 years ago.^[14] In contrast to subgenotype A1 that was dispersed by the slave trade,^[13] genotype E is rarely found outside Africa, except in individuals of African descent, further supporting a recent emergence following the forced migrations of slaves.

This paper serves to present the outcomes of our research on subgenotype A1 and genotype E, characterising the unique molecular features of these African strains, which most likely influence the natural history and the subsequent clinical manifestations of chronic HBV infection in Africa.^[15] The research was carried out in South Africa (SA), Zimbabwe, Kenya, Namibia, Nigeria and Sudan. The geographic distribution and prevalence of the HBV genotypes in these countries are shown in Fig. 1. Genotype A predominates in SA,^[11,12,16,17] Zimbabwe^[18] and Kenya^[19] and genotype E is common in Namibia^[20] and Nigeria.^[21] In the Sudan, three genotypes are described, genotypes A, D and E, and the lack of a predominant genotype most likely reflects Sudan's unique geographical location and the flux of peoples across its borders.^[22-24]

Genotype distribution in relation to clinical manifestation of infection

The predominance of genotype A and subgenotype A1 in SA, Zimbabwe and Kenya was found in blood donors, asymptomatic carriers of HBV and patients with cirrhosis and HCC.^[11,18,19] In SA HBV/HIV co-infected patients, 97% of the HBV isolates belong to subgenotype A1 and 3% to subgenotype D3.^[16] In Sudan, the genotype distribution varies according to the clinical manifestation

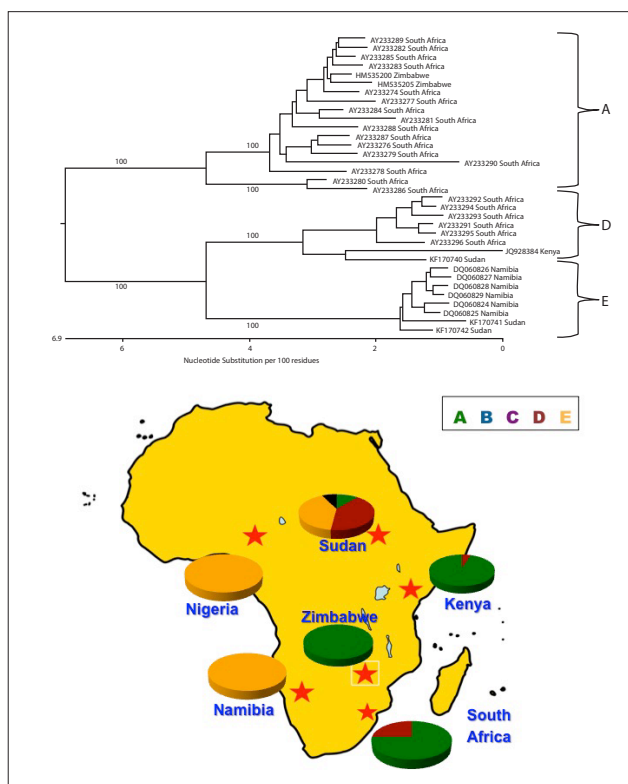


Fig. 1. The genotype distribution of HBV in Africa derived from our studies. The data were obtained by sequencing as follows: South Africa (23 full-length sequences of HBV isolates from asymptomatic carriers of the virus (ASCs) and fulminant (FH)/acute hepatitis (AH) cases);⁽¹¹⁾ Namibia (6 full-length HBV genomes from chronic hepatitis (CH) patients);⁽²⁰⁾ Zimbabwe (27 preS1/preS2/S sequences (nt 2854–835 numbering according to GenBank accession #AY233274) and 2 complete genomes from blood donors);^(18,21) Kenya (40 preS1/preS2/S sequences (nt 2854–835) from hepatocellular carcinoma (HCC), cirrhotic (CR), CH patients and ASCs);⁽¹⁹⁾ Nigeria (17 complete S/partial polymerase sequences (2535–1099 from EcoR1) of hepatitis B virus (HBV) isolated from HBV/HIV-co-infected individuals)⁽²¹⁾ and Sudan (53 preS1/preS2/S sequences (nt 2854–835) from HCC, CR, CH, AH patients and ASCs and HBV/HIV co-infected individuals).^(22,23) Insert shows an unrooted phylogenetic tree of 34 full genomes of HBV sequenced in our studies and established using neighbour-joining. Each sequence is designated by a GenBank accession number and the country of origin.

of the chronic HBV infection, with genotype E predominating in asymptomatic carriers and blood donors,^[23,24] whereas genotype D is found in those carriers with liver disease, including those patients with HCC.^[23] An opportunity to compare genotype D and E in a similar group of Sudanese^[23] revealed that patients infected with genotype E had a higher frequency of HBeAg-positivity and higher viral loads compared with those patients with HBV that were infected with genotype D.^[23] In a subsequent study, HBV/HIV co-infected patients had a higher frequency of genotype D/E, recombinant genotype A and intragroup divergence of genotype E compared with HBV mono-infected individuals.^[22]

Subgenotype A1

Africa is likely the origin of subgenotype A1, which spread globally to Asia and Latin America at approximately the same time.^[13] Our phylogeographic analysis including the unexpected finding of co-clustering of Somalian and Latin American strains and the

dispersal of subgenotype A1 from India to Haiti, correlates well with the historical evidence, which implicates trade and slave routes of dispersal of subgenotype A1, between the 9th and the 19th centuries AD.^[13]

Carriers of subgenotype A1 have characteristic clinical features, which includes lower HBV DNA levels in both the HBeAg-positive and HBeAg-negative phases. In a case-control study, HBV DNA levels in HBeAg-positive and HBeAg-negative subgenotype A1 carriers were significantly lower than those of subgenotype A2 or genotype D carriers.^[25] In HBV-infected individuals younger than 30 years of age the frequency of HBeAg-positivity is lower in those with subgenotype A1 compared with those infected with subgenotypes A2 or D.^[25] Perinatal transmission occurs at a relatively high rate in HBeAg-positive mothers compared with HBeAg-negative mothers,^[26] and since women of child-bearing age in Africa are usually younger than 30 years of age, and more often HBeAg-negative, perinatal transmission of subgenotype A1 is uncommon and vertical transmission is not an efficient mode of transfer. The most frequent mode of transmission of subgenotype A1 is horizontal and occurs via intrafamilial and to a lesser extent interfamilial route. Although the exact mode of horizontal transmission has not been elucidated, environmental, behavioural and cultural factors are likely to be contributory. Cultural practices such as scarification and tattooing and iatrogenic procedures including indiscriminate use of injections or non-sterilised syringes have all been implicated. In a study carried out with the South African National Blood Transfusion Service, we were able to show that subgenotype A1 can be transmitted horizontally by blood transfusion at very low viral levels (of 32 HBV DNA copies per 20 mL of plasma at a rate of 0.34 per million).^[27] Furthermore, HBV could not be detected in blood donors even though individual donor nucleic acid testing was performed.^[27]

By comparing the distribution of the genotypes in 111 southern African patients with HCC and an equivalent number of asymptomatic HBV carriers (ASCs), subgenotype A1 was shown to have an increased hepatocarcinogenic potential compared with subgenotype A2 and genotype D (relative risk of 4.5; 95% confidence interval 1.86 - 10.90).^[17] Moreover, the mean age of the HCC patients infected with genotype A (predominantly subgenotype A1) was 6.5 years younger than those infected with genotype non-A ($p < 0.05$).^[17] This confirms our earlier observation that the proportion of genotype A precore sequences (majority subgenotype A1) in HCC patients was significantly higher than those in ASCs.^[28] All precore sequences derived from tumorous and non-tumorous liver tissue samples had 1888^A - a characteristic of subgenotype A1.^[28]

By measuring the crude geometric means of M30, a systemic marker for apoptosis, SA chronic HBV carriers infected with subgenotype A1 had significantly higher levels of liver inflammation compared with patients infected with genotype D. Furthermore, patients with HCC also had significantly higher levels of M30 compared with ASCs.^[29]

The characterisation of subgenotype A1 may help us understand the mechanisms of tumorigenesis and may indeed help develop targets for both antiviral and anti-tumour therapies. In order to explain the high frequency of HBeAg-negativity and tumorigenic potential of subgenotype A1 our research focused on two aspects: functional and molecular characterisation.

Functional characterisation of subgenotype A1 using *in vitro* models

For this purpose, replication-competent plasmids of HBV subgenotypes A1, A2 and D3 were constructed with authentic endogenous promoters.^[30] Following transfection of Huh7 cells,

subgenotype A1 plasmids replicated at lower levels than subgenotype A2,^[30,31] which reflects the difference observed in patients infected with different subgenotypes.^[25] This is consistent with the observations of others in transfection experiments^[31] leading to the intracellular accumulation of replicative intermediates and HBcAg,^[30] which may play a role in inducing liver damage.^[31] This has been implicated in hepatocarcinogenesis and is supported by *in vitro* experiments, which demonstrated that Huh7 cells transfected with subgenotype A1 showed greater endoplasmic reticulum (ER) stress compared with cells transfected with subgenotypes A2 and D3. Moreover, there was an earlier and prolonged activation of the unfolded protein response (UPR) as seen by the high activity of three ER-localised transmembrane transducers: double-stranded RNA-dependent protein kinase-like ER kinase, activating transcription factor 6 and inositol-requiring enzyme 1. In addition, cells transfected with subgenotype A1 have the highest level of apoptosis.^[32]

Basic core promoter (BCP)/precore mutations and variations of subgenotype A1

Subgenotype A1 develops different mutations in the BCP/precore region when compared with subgenotypes A2 and D3.^[33,34] There are three levels at which these mutations/variations can affect HBeAg expression.

Firstly, at the transcriptional level, the BCP mutations 1762^T1764^A have an effect on the transcription of the precore mRNA, which results in the reduction of HBeAg expression. The double mutation is highly prevalent in HBV isolates from patients with HCC. Our earlier studies showed that 66% of patients with HCC had mutations 1762^T1764^A in the BCP compared with only 11% of ASCs.^[35] This observation has been confirmed by numerous subsequent studies regardless of the HBV genotype. The double BCP mutation has been shown to be an independent risk factor for the development of HCC in genotypes B and C.^[36]

Secondly, variations at positions 1809 - 1812 from the *EcoRI* site in the Kozak sequence of the precore/core open reading frame, characteristic of subgenotype A1, have an effect on HBeAg expression at the translational level.^[37] Instead of ¹⁸⁰⁹GCAC¹⁸¹², which is present in subgenotypes A2 and D3, subgenotype A1 has ¹⁸⁰⁹TCAT¹⁸¹².^[34] This variation converts the Kozak region from an optimal to a suboptimal translational context^[11] and results in the decreased translation of HBeAg by a ribosomal leaky scanning mechanism.^[37] Compared with subgenotypes A2 and D3, transfection with subgenotype A1 leads to a lower expression of the precore/core precursor in the secretory pathway and a higher co-localisation in the nucleus.^[32] The reduction in HBeAg levels is comparable with that observed in the presence of 1762^T1764^A and when occurring together, the Kozak and BCP 1762^T1764^A mutations reduce HBeAg expression in an additive manner.^[37] An additional mutation at position 1810 severely impairs HBeAg expression.^[37] The reduction in HBeAg expression may contribute to the early loss of HBeAg in individuals infected with subgenotype A1.^[25]

Thirdly, a G to T transversion at position 1862 in the precore region occurs frequently in subgenotype A1, and is more common in HBeAg-negative than in HBeAg-positive South African carriers,^[28,38] which affects HBeAg expression at the post-translational level. This mutation results in a valine to phenylalanine substitution in the -3 position of the signal peptide cleavage site at position 19 of the precursor protein. Phenylalanine is an aromatic amino acid which interferes with signal peptide cleavage.^[39] When this mutation was introduced into a genotype D plasmid driven by a cytomegalovirus promoter, it resulted in a 54% reduction in the secretion of HBeAg relative to the wild-type and to the subsequent formation of aggresomes.^[40] In the context

of a subgenotype A1 backbone, this mutation diminishes HBeAg expression but at a lower degree (22%). The mutant was found to lead to the accumulation of the HBeAg precursor protein in the ER and ER-Golgi intermediate compartment (ERGIC) and this accumulation resulted in an earlier activation of the three UPR pathways. It did not however lead to an increase in apoptosis.^[32] HBV strains with ^G1862^T have been isolated from sites of HCC tumors but not from adjacent non-tumorous liver tissue.^[28]

As a result patients, infected with subgenotype A1 with characteristic suboptimal Kozak sequence preceding the precore start codon, together with 1762^T1764^A and 1862^T mutants, have severely diminished levels of HBeAg. The reduction or absence of HBeAg in the serum results in an immune response driven directly to hepatocytes, which together with the increased ER stress, results in liver damage and thus contributes to the higher hepatocarcinogenic potential of the A1 subgenotype.^[34]

In addition to the characteristic ¹⁸⁰⁹TCAT¹⁸¹² in the precore Kozak sequence, subgenotype A1 has 1888^A, differentiating it from subgenotype A2 and other genotypes that have 1888^G, results in a positive regression coefficient for subgenotype A1 and a negative one for subgenotype A2.^[41] This transition introduces an out-of-frame AUG, creating an overlapping upstream open reading frame (uORF), terminating five nucleotides downstream from the core AUG.

This uORF can potentially be translated into a seven amino acid peptide. When Huh7 cells were transfected with reporter plasmids^[42] and the introduction of uORF resulted in an 18.75% reduction in core gene expression. When the suboptimal Kozak sequence of the 1888 AUG was replaced with an optimal one, the reduction was enhanced (64.84%). By increasing the distance between the stop of the overlapping uORF and the core AUG by a minimum of 15 nucleotides, core/GFP expression was almost doubled, indicating that stalling of ribosomes at the stop of the uORF may interfere with initiation at the core AUG through steric hindrance. Our findings indicate that the ^G1888^A mutation interferes with initiation at the downstream 1901 core AUG decreasing core protein translation. This may account for the relatively low viral loads seen in individuals infected with subgenotype A1.^[42]

Pre-S1/pre-S2/S deletion mutants in subgenotype A1

Eighteen SA HCC patients infected with subgenotype A1 were studied and 72% had HBV pre-S gene deletions. The most prevalent deletions were deletions across pre-S1/pre-S2, pre-S2 initiation codon mutations with internal deletions and S gene nonsense mutations.^[43] Similar observations were made in HBV strains isolated from Indian HCC patients infected with subgenotype A1.^[44] The pre-S2 start codon MIT/I mutation was unique to genotype A strains and occurred at a statistically significantly higher frequency in isolates from patients with HCC.^[44] Both experimental and clinical studies provide strong evidence for a link between these mutants and the pathogenesis of HCC. A meta-analysis of 43 studies that evaluated approximately 11 500 HBV-infected patients, showed that infection with pre-S deletion mutants is associated with a 3.77-fold increased risk of HCC.^[45] Moreover, a prospective study revealed a predictive value of pre-S mutants in the development of HCC and pro-oncogenic role of mutated envelope proteins.^[46]

Although most of our work has focused on subgenotype A1, we have now initiated work on the molecular and functional characterisation of genotype E, which is an African strain with unique characteristics.

Genotype E

Genotype E has the unique serological subtype *ayw4*, a genomic

length of 3 212 nucleotides and can be differentiated from genotypes A to D, F, H and I, by a 3-nucleotide deletion in the pre-S1 region.^[9] In addition, we have identified another unique feature of genotype E strains: the introduction of another start codon Met⁸³ in the pre-S1 region that may result in the translation of an elongated middle hepatitis B surface protein (MHBs).^[20]

Western Africa represents the only major region in the world where HBV is still hyperendemic and the predominant genotype prevailing there is genotype E.^[1] In Sudan, where both genotypes D and E are prevalent, it was possible to compare the clinical features of patients infected with these two genotypes. Patients with liver disease^[23] and blood donors^[24] had viral loads that were significantly higher in genotype E-infected patients compared with genotype D-infected patients and furthermore patients infected with genotype E were more likely to be HBeAg-positive. The G1896^A mutation accounts for the HBeAg-negativity in non-A genotypes and this mutation introduces a stop-codon in the HBeAg precursor, which leads to its truncation and to the non-expression of the mature HBeAg.^[47] The region that codes for HBeAg overlaps pregenomic RNA, which requires to be folded into a secondary structure known as the encapsidation signal (ϵ). In genotype A, there is an 1858^C, which prevents the development of 1896^A since this would disrupt Watson-Crick C-G base pair and lead to the destabilisation of ϵ . On the other hand, in genotypes such as D and E, which have an 1858^T, the G1896^A mutation results in the stabilisation of ϵ with the conversion of a wobble to a stable Watson-Crick T-A pair. Presently it is unclear why G1896^A is positively associated with genotype D and negatively associated with genotype E,^[41] even though both genotypes have 1858^T. This lack of association of G1896^A with genotype E might explain the high frequency of HBeAg-positivity in individuals infected with genotype E. Moreover, a high frequency of HBeAg-positivity in mothers infected with genotype E would account for significant perinatal transmission^[26] and possibly explain the high prevalence and geographical location in western Africa.

Since HBeAg is small it is able to cross the placenta easily and elicits HBe/HBcAg-specific T helper cell tolerance *in utero*.^[48] As a result, babies born to HBeAg-positive mothers in Africa with genotype E have high chronicity rates compared with those born to HBeAg-negative mothers with genotype E.^[48,49]

The higher HBeAg-positivity described in individuals infected with genotype E might confer tolerance and a milder clinical manifestation of the disease than genotype D, where HBeAg-positivity was lower. This could explain the higher prevalence of genotype E in Sudanese blood donors,^[24] whereas genotype D is more prevalent in those patients with liver disease.^[23]

In the Sudan, although pre-S deletion mutants were found in both genotype D and genotype E they were only found in HCC patients infected with genotype E. In genotype D patients they were found in ASC, cirrhotics and chronic hepatitis patients^[23] and the significance of this is unknown.

Genotypes in HBV/HIV co-infection and occult HBV infection

Of the 36 million adults and children living with HIV globally, 27 million reside in sub-Saharan Africa (SSA). According to WHO, the HBV/HIV co-infected rate in SSA is highest in West Africa (74%) and decreases in SSA countries in eastern Africa (48%) and in southern Africa (41%), with an average exposure rate of 44% in SSA.^[50]

Co-infected HBV/HIV individuals have a higher mortality and morbidity compared with individuals who are mono-infected with

either HIV or HBV. The progression of chronic HBV to cirrhosis, end-stage liver disease (ESLD), and HCC is more rapid in HIV-positive individuals than those with HBV alone.^[51] Immunosuppression due to HIV infection causes loss of anti-HBs and leads to HBV reactivation. In addition, HBV vaccination results in a poor antibody response due to the immunocompromised state and negatively impacts on HIV outcomes,^[52] with antiretroviral therapy (ART) contributing to the increasing mortality attributed to HBV-associated ESLD.^[53]

We investigated HBV/HIV co-infection in ART-naïve adults in SA^[54] and Sudan. Approximately 74% had serological markers for chronic HBV infection and of the 298 South Africans tested, 71 (24%) were HBV DNA-positive. The number of lifetime sexual partners was the only factor differentiating HBV DNA-positive from HBV DNA-negative individuals, implicating sexual transmission for either HBV or HIV. Of the 71 who were HBV DNA-positive, 26 (9%) were HBsAg-positive and 45 (15%) were HBsAg-negative. Only 7% of the HBsAg-negative individuals were true occult infections with viral loads of less than 200 IU/ml^[55] and the remaining 93% had higher viral loads defined as HBsAg-covert.^[54] The individuals who were HBsAg-negative did not differ significantly from the HBsAg-positive in terms of viral load and CD4 counts. HIV-infected individuals were predominantly infected with HBV subgenotype A1.^[16] We identified mutations that could account for both the HBeAg- and HBsAg-negativity. HBV isolates from HIV-infected individuals had pre-S deletion mutants that were identical to those isolated from HCC patients. In a study we carried out in collaboration with Chinese colleagues, we showed that 1762^T1764^A or pre-S deletions occurred more frequently (44.3% and 23%, respectively) in isolates from HBV/HIV co-infected compared with HBV mono-infected individuals (21.3% and 4.9%, respectively) ($\chi^2=7.290$, $p<0.05$; $\chi^2=8.270$, $p<0.05$). In the SA study, 10% of patients had drug resistance mutations prior to the initiation of ART.^[16]

In the Sudanese study 358 HIV-infected individuals were tested: 96 (63%) had exposure to HBV and 27% were HBV DNA-positive. Risk factors for HBV DNA-positivity included a previous history of blood transfusions, dental procedures, jaundice and WHO classification of AIDS of 3 to 4.^[56] Of the 96 HBV DNA-positive individuals, 42 were HBsAg-positive (11.7%) and 54 HBsAg-negative (15.1%), with higher viral loads in the HBsAg-positive individuals. A higher proportion of the HBsAg-negative individuals (65%) were true HBsAg-occult and the remaining 35% were HBsAg-covert^[56] which contrasts with the SA study described above.^[54] The course of HBV and/or HIV infection and in genotypes (subgenotype D and E v. subgenotype A1) may contribute to these differences. Compared with HBV mono-infected individuals, the frequency of subgenotype D/E recombinant and subgenotype A was higher in HBV/HIV co-infected Sudanese, as was the intragroup divergence of genotype E.^[22] Isolates from two patients infected with genotype E revealed pre-S deletion mutants, which were similar to those seen in HIV-co-infected patients from SA and from patients with HCC.

In both the South African and Sudanese studies, we found that approximately 25% of HIV-infected individuals were co-infected with HBV and 15% were HBsAg-negative. Therefore HBsAg serological testing under-estimates the presence of HBV in HIV co-infected individuals. In the case of the South Africans infected predominantly with subgenotype A1, the HBsAg-covert infections did not differ from overt infections in terms of viral load. HBV from HIV co-infected individuals from both cohorts had pre-S deletion mutants identical to those isolated from HCC patients. Drug resistance mutations in HBV isolates can occur prior to the initiation of ART.

Conclusions

Strains of HBV commonly found in Africa differ from those outside Africa both in terms of molecular and functional characteristics and therefore studies conducted outside Africa cannot necessarily be extrapolated to the African continent. Although considerable progress has been made in Africa with regard to HBV genotyping research, there is no room for complacency. Unprecedented human migrations, currently taking place from Africa, are contributing to the dispersion of HBV from a continent where HBV is endemic to low endemicity areas. Introduction of the virus to regions of the world where universal vaccination is not generally practised will hamper efforts to achieve the WHO target of global eradication of HBV as a public health threat by 2030.

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Conflicts of interest. None.

- Kramvis A, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. *Hepatol Res* 2007;37(s1):S9-S19. <https://doi.org/10.1111/j.1872-034X.2007.00098.x>
- Parkin DM, Sitas F, Chirenje M, Stein L, Abratt R, Wabinga H. Part I: Cancer in Indigenous Africans—burden, distribution, and trends. *Lancet Oncol* 2008;9(7):683-692. [https://doi.org/10.1016/S1470-2045\(08\)70175-X](https://doi.org/10.1016/S1470-2045(08)70175-X)
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011;61(2):69-90. <https://doi.org/10.3322/caac.20107>
- The Cancer Atlas. Cancer in Sub-Saharan Africa. <http://canceratlas.cancer.org/the-burden/cancer-in-sub-saharan-africa/> (accessed 4 June 2017).
- Moore SW, Miller AJ, Hadley GP, et al. Hepatocellular carcinoma and liver tumors in South African children: A case for increased prevalence. *Cancer* 2004;101(3):642-649. <https://doi.org/10.1002/cncr.20398>
- Kew MC. Hepatocellular carcinoma in African Blacks: Recent progress in etiology and pathogenesis. *World J Hepatol* 2010;2(2):65-73. <https://doi.org/10.4254/wj.v2.i2.65>
- Kew MC, Asare GA. Dietary iron overload in the African and hepatocellular carcinoma. *Liver Int* 2007;27(6):735-741. <https://doi.org/10.1111/j.1478-3231.2007.01515.x>
- Kew MC. Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. *Liver Int* 2003;23(6):405-409.
- Kramvis A. Genotypes and genetic variability of hepatitis B virus. *Intervirol* 2014;57(3-4):141-150. <https://doi.org/10.1159/000360947>
- Kramvis A, Kew M, François G. Hepatitis B virus genotypes. *Vaccine* 2005;23(19):2409-2423. <https://doi.org/10.1016/j.vaccine.2004.10.045>
- Kimbi GC, Kramvis A, Kew MC. Distinctive sequence characteristics of subgenotype A1 isolates of hepatitis B virus from South Africa. *J Gen Virol* 2004;85(Pt 5):1211-1220. <https://doi.org/10.1099/vir.0.19749-0>
- Bowyer SM, van Staden L, Kew MC, Sim JG. A unique segment of the hepatitis B virus group A genotype identified in isolates from South Africa. *J Gen Virol* 1997;78(Pt 7):1719-1729. <https://doi.org/10.1099/0022-1317-78-7-1719>
- Kramvis A, Paraskevis D. Subgenotype A1 of HBV – tracing human migrations in and out of Africa. *Antivir Ther* 2013;18(3 Pt B):513-521. <https://doi.org/10.3851/IMP2657>
- Andernach IE, Hunewald OE, Muller CP. Bayesian inference of the evolution of HBV/E. *PLoS One* 2013;8(11):e81690. <https://doi.org/10.1371/journal.pone.0081690>
- Kramvis A, Kew MC. Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. *J Viral Hepat* 2005;12(5):456-464. <https://doi.org/10.1111/j.1365-2893.2005.00624.x>
- Makondo E, Bell TG, Kramvis A. Genotyping and molecular characterization of hepatitis B virus from human immunodeficiency virus-infected individuals in Southern Africa. *PLoS ONE* 2012;7(9):e46345. <https://doi.org/10.1371/journal.pone.0046345>
- Kew MC, Kramvis A, Yu MC, Arakawa K, Hodgkinson J. Increased hepatocarcinogenic potential of hepatitis B virus genotype A in Bantu-speaking sub-saharan Africans. *J Med Virol* 2005;75(4):513-521. <https://doi.org/10.1002/jmv.20311>
- Gulube Z, Chairara M, Kew M, Tanaka Y, Mizokami M, Kramvis A. Molecular characterization of hepatitis B virus isolates from Zimbabwean blood donors. *J Med Virol* 2011;83(2):235-244. <https://doi.org/10.1002/jmv.21954>
- Ochwoto M, Chauhan R, Gopalakrishnan D, et al. Genotyping and molecular characterization of hepatitis B virus in liver disease patients in Kenya. *Infect Genet Evol* 2013;20:103-110. <https://doi.org/10.1016/j.meegid.2013.08.013>
- Kramvis A, Restorp K, Norder H, Botha JF, Magnus LO, Kew MC. Full genome analysis of hepatitis B virus genotype E strains from South-Western Africa and Madagascar reveals low genetic variability. *J Med Virol* 2005;77(1):47-52. <https://doi.org/10.1002/jmv.20412>
- Grant J, Agbaji O, Kramvis A, et al. Hepatitis B virus sequencing and liver fibrosis evaluation in HIV/HBV co-infected Nigerians. *Trop Med Int Health* 2017;22(6):744-754. <https://doi.org/10.1111/tmi.12873>
- Yousif M, Mudawi H, Hussein W, et al. Genotyping and virological characteristics of hepatitis B virus in HIV-infected individuals in Sudan. *Int J Infect Dis* 2014;29:125-132. <https://doi.org/10.1111/tmi.12873>
- Yousif M, Mudawi H, Bakhtiet S, Glebe D, Kramvis A. Molecular characterization of hepatitis B virus in liver disease patients and asymptomatic carriers of the virus in Sudan. *BMC Infect Dis* 2013;13:328. <https://doi.org/10.1186/1471-2334-13-328>
- Mahgoub S, Candotti D, El Ekiaby M, Allain JP. Hepatitis B virus (HBV) infection and recombination between HBV genotypes D and E in asymptomatic blood donors from Khartoum, Sudan. *J Clin Microbiol* 2011;49(1):298-306. <https://doi.org/10.1128/JCM.00867-10>
- Tanaka Y, Hasegawa I, Kato T, et al. A case-control study for differences among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. *Hepatology* 2004;40(3):747-755. <https://doi.org/10.1002/hep.20365>
- Kramvis A. The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. *Rev Med Virol* 2016;26(4):285-303. <https://doi.org/10.1002/rmv.1885>
- Vermeulen M, Dickens C, Lelie N, et al. Hepatitis B virus transmission by blood transfusion during 4 years of individual-donation nucleic acid testing in South Africa: estimated and observed window period risk. *Transfusion* 2012;52:880-892. <https://doi.org/10.1111/j.1537-2995.2011.03355.x>
- Kramvis A, Kew MC, Bukofzer S. Hepatitis B virus precore mutants in serum and liver of Southern African Blacks with hepatocellular carcinoma. *J Hepatol* 1998;28(1):132-141.
- Viana RV. The relationship between hepatitis B virus and apoptosis in humans and in a transgenic mouse model. *Johannesburg: University of the Witwatersrand*; 2012.
- Bhoola NH, Reumann K, Kew MC, Will H, Kramvis A. Construction of replication competent plasmids of hepatitis B virus subgenotypes A1, A2 and D3 with authentic endogenous promoters. *J Virol Methods* 2014;203:54-64. <https://doi.org/10.1016/j.jviromet.2014.03.015>
- Sugiyama M, Tanaka Y, Kato T, et al. Influence of hepatitis B virus genotypes on the intra- and extracellular expression of viral DNA and antigens. *Hepatology* 2006;44(4):915-924. <https://doi.org/10.1002/hep.21345>
- Bhoola NH, Kramvis A. Hepatitis B e antigen expression by hepatitis B virus subgenotype A1 relative to subgenotypes A2 and D3 in cultured hepatocellular carcinoma (Huh7) cells. *Intervirol* 2016;59(1):48-59. <https://doi.org/10.1159/000446240>
- Bell TG, Kramvis A. Mutation reporter tool: An online tool to interrogate loci of interest, with its utility demonstrated using hepatitis B virus. *Virol J* 2013;10(1):62. <https://doi.org/10.1186/1743-422X-10-62>
- Kramvis A, Kew MC. Molecular characterization of subgenotype A1 (subgroup Aa) of hepatitis B virus. *Hepatol Res* 2007;37(s1):S27-32. <https://doi.org/10.1111/j.1872-034X.2007.00100.x>
- Baptista M, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. *Hepatology* 1999;29(3):946-953. <https://doi.org/10.1002/hep.510290336>
- Liu CJ, Chen BF, Chen PJ, et al. Role of hepatitis B virus precore/core promoter mutations and serum viral load on noncirrhotic hepatocellular carcinoma: a case-control study. *J Infect Dis* 2006;194(5):594-599. <https://doi.org/10.1086/505883>
- Ahn SH, Kramvis A, Kawai S, et al. Sequence variation upstream of precore translation initiation codon reduces hepatitis B virus e antigen production. *Gastroenterology* 2003;125(5):1370-1378.
- Kramvis A, Bukofzer S, Kew MC, Song E. Nucleic acid sequence analysis of the precore region of hepatitis B virus from sera of southern African black adult carriers of the virus. *Hepatology* 1997;25(1):235-240. <https://doi.org/10.1002/hep.510250143>
- Nielsen H, Engelbrecht J, Brunak S, von Heijne G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng* 1997;10(1):1-6.
- Chen CY, Crowther C, Kew MC, Kramvis A. A valine to phenylalanine mutation in the precore region of hepatitis B virus causes intracellular retention and impaired secretion of HBe-antigen. *Hepatol Res* 2008;38(6):580-592. <https://doi.org/10.1111/j.1872-034X.2007.00315.x>
- Kramvis A, Arakawa K, Yu MC, Nogueira R, Stram DO, Kew MC. Relationship of serological subtype, basic core promoter and precore mutations to genotypes/subgenotypes of hepatitis B virus. *J Med Virol* 2008;80(1):27-46.
- Kimbi GC, Kew MC, Kramvis A. The effect of the G1888A mutation of subgenotype A1 of hepatitis B virus on the translation of the core protein. *Virus Res* 2012;163(1):334-340. <https://doi.org/10.1016/j.virusres.2011.10.024>
- Skelton M. Characterization of mutants and splice variants of hepatitis B virus isolated from South African Black hepatocellular carcinoma patients. *Johannesburg: University of the Witwatersrand*; 2009.
- Gopalakrishnan D, Keyter M, Shenoy KT, et al. Hepatitis B virus subgenotype A1 predominates in liver disease patients from Kerala, India. *World J Gastroenterol* 2013;19(48):9294-9306. <https://doi.org/10.3748/wjg.v19.i48.9294>
- Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, et al. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: A meta-analysis. *J Natl Cancer Inst* 2009;101(15):1066-1082. <https://doi.org/10.1093/jnci/djp180>
- Chen CH, Hung CH, Lee CM, et al. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. *Gastroenterology* 2007;133(5):1466-1474. <https://doi.org/10.1053/j.gastro.2007.09.002>
- Carman WF, Jacyna MR, Hadziyannis S, et al. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989;2(8663):588-591.
- Milich D, Liang TJ. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. *Hepatology* 2003;38(5):1075-1086. <https://doi.org/10.1053/jhep.2003.50453>
- Mulders MN, Venard V, Njayou M, et al. Low genetic diversity despite hyperendemicity of hepatitis B virus genotype E throughout West Africa. *J Infect Dis* 2004;190(2):400-408. <https://doi.org/10.1086/421502>
- Matthews PC, Geretti AM, Goulder PJ, Klennerman P. Epidemiology and impact of HIV coinfection with hepatitis B and hepatitis C viruses in Sub-Saharan Africa. *J Clin Virol* 2014;61(1):20-33. <https://doi.org/10.1016/j.jcv.2014.05.018>
- Thio CL, Seaberg EC, Skolasky R Jr, et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). *Lancet* 2002;360(9349):1921-1926.
- Chun HC, Roediger MP, Hullsiek KH, et al. Hepatitis B virus coinfection negatively impacts HIV outcomes in HIV seroconverters. *J Infect Dis* 2011;205(2):185-193. <https://doi.org/10.1093/infdis/jir720>
- Thomas DL. Growing importance of liver disease in HIV-infected persons. *Hepatology* 2006;43(2 Suppl 1):S221-S229. <https://doi.org/10.1002/hep.21033>
- Bell TG, Makondo E, Martinson NA, Kramvis A. Hepatitis B virus infection in human immunodeficiency virus infected southern African adults: occult or overt—that is the question. *PLoS One* 2012;7(10):e45750. <https://doi.org/10.1371/journal.pone.0045750>
- Raimondo G, Allain JB, Brunetto MR, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008;49(4):652-657. <https://doi.org/10.1016/j.jhep.2008.07.014>
- Mudawi H, Hussein W, Mukhtar M, et al. Overt and occult hepatitis B virus infection in adult Sudanese HIV patients. *Int J Infect Dis* 2014;29:65-70. <https://doi.org/10.1016/j.ijid.2014.07.004>