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Hepatitis B Virus ~ a millionth of the human genome

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2013-10-03
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Kramvis, Anna. (2013). Hepatitis B Virus ~ a millionth of the human genome. [Inaugural lecture, University of the Witwatersrand, Johannesburg]. WIReDSpace. https://hdl.handle.net/10539/49259

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Inaugural Lecture
Professor Anna Kramvis
3rd October 2013

Thank you for the kind introductions, Professor Kupe and Professor Fonn.

Professor Kupe (Deputy Vice-Chancellor – Finance)
Professor Fonn (Acting Dean – Faculty of Health Sciences)
Professor Kramer (Assistant Dean Research and Postgraduate Support)
Professor Cooper (Acting Head of the School of Clinical Medicine)
Professor Naicker (Head of Internal Medicine)

Good evening ladies and gentlemen, colleagues, students, family and friends, I am honoured and humbled by your presence here this evening.

My journey to this podium has been wonderful and interesting and I have been fortunate to have been accompanied by people who inspired and supported me along the way. Tonight I want to pay tribute to them.

It was my parents, Vassili and Melpo Tragellis who first recognized by academic leanings, gave me the opportunities and encouraged me to pursue my dreams. I am blessed to have my Father in the audience tonight. Ευχαριστώ, thank you Daddy. My mother always told me, that from a very young age, I was fascinated by small things but even she could not have anticipated the small size of the object/subject that has captured my interest in the last 18 years or so.

Hepatitis B Virus ~ a millionth of the human genome

~the smallest DNA virus infecting man.

This slide illustrates the size of HBV relative to other viruses, we can align 36 000 virus particles across the diameter of a pinhead!

In the next 40 minutes or so, I would firstly like to deconstruct the title of my talk and in the process give you a crash course in hepatology, molecular biology, virology and Greek and next I would like to share with you some of the major highlights of our research, which include:

- Subgenotype A1:
  - Identification
  - Epidemiology in and out of Africa
  - Molecular and functional characterization
  - Co-infection with HIV

The central role of the **liver** in our body is reflected by the origin of the names used to describe this important organ. The ancient Greek word for liver **"hepar" [*ήπαρ*]** is derived from the word "pleasure" because in Ancient Greece the liver was considered to be the seat of the soul and human feelings. Figs **(*σύκα* - syka)** were fed to animals, in order to fatten their livers, which were used to prepare many delicacies, known as ***συκωτόν ήπαρ*** (sykoton hepar), which were equivalent to today's liver pate or *foie gras*. With time, the term "hepar" was dropped, giving rise to ***συκώτι*** **(sykoti),** the modern Greek word for liver. The original ancient Greek name *hepar* however has been retained in medical terms: ***hepatology*** – the study of liver and liver disease, **hepatitis** – inflammation of the liver; **hepatocellular carcinoma** – liver cancer.

The primary **function of the liver** is to filter almost everything we consume. It helps to protect us against infection and removes bacteria and other toxins from the blood. The liver is responsible for the storage of energy and vitamins such as A, B12, D and K, which we require for our activities. The liver also controls the levels of blood sugar, cholesterol as well as several hormones and enzymes.

The liver is able to regenerate itself. In Greek mythology, when **Prometheus** (*Προμηθεύς*) stole the secret of fire from the gods for human use, he was punished by being bound to a rock, and his liver was eaten by an eagle, on a daily basis, only to grow back overnight.

The most notable sign of liver disease or **hepatitis** is jaundice, a yellow discolouration of the skin and eyes. This is caused by the deposit of bile pigments, which the damaged liver has been unable to eliminate from the blood. Other symptoms include: weakness, tiredness, muscle aches, mild fever, nausea, poor appetitie, dark urine and pale stool. These symptoms are often overlooked or dismissed as flu and often people have hepatitis without being aware of it. It was **Hippocrates** (460 to 375 BC) who was the first to document the symptoms of hepatitis and use the term *icterus* in his *Corpus Hippocraticum* (Hippocratic Collection).

Although hepatitis can be caused by toxins, including alcohol, a number of medications and by autoimmune diseases, the major cause of hepatitis is infection with viruses. The term **viruses** is derived from the Latin meaning toxin or poison, coined in 25 A.D. In the 1880s scientists, including **Pasteur**, recognized that bacteria could cause disease and that these cellular organisms could be removed by filtration. However, at the beginning of the 20th century, it became increasingly evident that certain infections could be transmitted by serum, even after filtration and this led to the conclusion by **Walter Reed** in 1902 that diseases could be caused by ultramicroscopic organisms. It was not until the development of the electron microscope in the 1930s that the filterable organisms could be visualized and **virology**, the study of viruses, became established as a discipline.

I was introduced to virology in my undergraduate years by **Professor Helen Garnett,** who encouraged me to pursue my postgraduate studies because she

believed in me before I did. She also taught me tenacity to face the frequent disappointments of science. I am grateful and indebted to her. When Professor Garnett gave her inaugural lecture in 1979, I remember thinking that I wanted to have just as many shoes as she did, and also to be as good a scientist and mentor as she was. I think I have achieved the first objective and I am working on the other two! For my PhD, I worked with cytomegalovirus, a DNA virus four times as large as HBV. I established a tissue culture system for vervet monkey bone marrow stromal cells and showed that cytomegalovirus can replicate in these cells and interfere with haemopoiesis, another Greek word, meaning the production of blood.

Today we know that there are at least **five hepatitis viruses**, named alphabetically – A to E, shown here in a clockwise direction but not to scale. Hepatitis A and E, which only cause an acute infection, are transmitted enterically, by the foecal-oral route. In other words, through contaminated food and water. Hepatitis B, C and D, which cause both acute and chronic infections, are blood-borne pathogens and can be transmitted parenterally, by blood products, sexual contact and from mother-to-child at the time of birth. The five hepatitis viruses are unrelated and belong to different **families** and differ in their genomes and structure. Even though our research team has worked with hepatitis viruses A to D, our major focus has been hepatitis B virus. HBV is the only hepatitis virus with a DNA genome

Although it was known for a while that DNA was the carrier of the hereditary information of organisms, its structure was only elucidated in 1953, when Watson and Crick constructed a model of DNA using the X-ray crystallography results of Rosalind Franklin and Maurice Wilkins. I found that the best explanation for the structure of DNA was not in the seminal paper they published in Nature but in a letter the Francis Crick wrote to his son, Michael before submitting to Nature. This letter has recently been sold for 6 million dollars. I will read you some excerpts, because it is poignant and communicates the sense of excitement and emotion of the “eureka” moment!!

The **central dogma** of molecular biology, explains how DNA acts as a code and how information flows from one polymer to another, that is from nucleic acids (DNA or RNA) to proteins. The synthesis of RNA using DNA as a template is known as **transcription**. Proteins are synthesized using the information provided by the RNA by **translation**. This takes place when the RNA code is read by tiny protein-assembly machines known as **ribosomes.** Each ribosome works along the RNA, reading the code from “start” to “stop”, selecting the correct amino-acid building blocks to form the protein. The sequence between the start and stop codons is known as an **open reading frame** (or ORF).

Up to 1970s it was believed that this flow of information went in one direction. This was overturned when it was discovered by Temin and Baltimore that RNA could be copied into DNA by **reverse transcription**. This mechanisms is used by retroviruses, RNA viruses, including the human immunodeficiency virus (HIV), which causes AIDS and by pararetroviruses, DNA viruses including HBV that replicate by way of an RNA intermediate.

The DNA that provides the genetic code for human life was published in 2003 by the **Human Genome Project.** It consists of 3 billion base pairs and encodes 20 000 to 25 000 genes. If we had to write the human genome out, it will fill ~1 million pages, which is the equivalent of 1 600 books and if we could read at a rate of 8 base pairs per second it would take us 34 years to read the sequence of the human genome!

In contrast, the HBV genome, which is **~3 200 nucleotides** long will fit in **one** page. HBV is the prototype member of the family *Hepadnavirus* that can infect both mammals and birds.

This genome is extremely efficient, well organized and economical, which is probably why HBV has managed to survive for thousands of years, to jump across species and adapt itself in different organisms. Even with the use of a very successful vaccine and antiviral drugs, it still manages to escape both the immune response and different treatment modalities, respectively. Despite suffering from its success, it is impossible for us humans not to admire the capabilities of these 3200 base pairs! This genome has generated so many questions that is keeping at least one scientist, for each of its nucleotides, busy.

Despite having a relatively small genome it displays amazing sequence heterogeneity because the reverse transcriptase/polymerase lacks proofreadig ability. Currently HBV is classified into at least 9 genotypes and 32 subgtenotypes, which are characterized by distinct geographical distribution both globally and locally. Our work and those of others have shown that genotypes/subgenotypes can affect the molecular characteristics of the virus, clinical manifestation of HBV infection in hosts, and response to antiviral therapies and vaccination

The 3 200 bps form partially double-stranded HBV genome, which contains four partly overlapping open reading frames (ORFs) that encode for 7 proteins:
P encoding the polymerase (reverse transcriptase), **S** for the three forms of envelope proteins (small, medium and large HBsAg), **C** that encodes for core protein (HBcAg). These proteins form the **infectious virion** schematically illustrated here. In addition, the genome codes for two additional proteins that do not form part of the virion: the **X** protein that has transactivating activity and **HBeAg.**

HBeAg is not required for virion production. Clinically it has been used as a marker of viral replication, infectivity, and response to antiviral therapy. HBeAG-positive patients have higher viral loads than those not expressing HBeAg. HBeAg has been implicated in establishing immune tolerance and chronic infection. The presence of HBeAg in the mother at the time of birth will determine whether mother to child transmission will occur. A baby born to a HBeAg-positive mother will have tolerance against the virus and the infection will become chronic. On the the other hand, the baby born to a HBeAg-negative mother will mount an immune response against the virus and the infection will be acute and will not persist.

The prevalence of hepatitis B in a community can be estimated by the proportion of the population who are HBsAg-positive carriers. Globally there are at least 240 million carriers of HBV and **carrier rates** vary widely. Sub-Saharan Africa and south-east Asia are areas of hyperdendemicity and there is a correspondingly high annual incidence of **hepatocellular carcinoma**.

In 1994 I joined the **Molecular Hepatology Research Unit** (MHRU) as a senior research officer, shortly after it was established by Professor Kew. The primary aim of the MHRU was to investigate at a molecular level, the mechanisms involved in the pathogenesis of hepatocellular carcinoma in Southern Africans, in particular in relation to HBV.

What impressed me, more than anything else about Prof Kew, was his contagious enthusiasm and passion for research, which he transmitted to me very early on. His exemplary work ethic made it very easy for me to work by his side for close to 14 years. Together we have published 39 papers, making me, his most frequent co-author, an honour I am very privileged to have. His wise and sound advice will always prove useful. I thank him tonight.

Professor Kew of course introduced me to HBV and taught me much including how to amplify DNA using the polymerase chain reaction (PCR) and **manual sequencing**, which was quite a process. In those early days, which were not that long ago, one had to run a PCR, do a sequencing reaction using radioactivity, which required accurate timing and correct maintenance of the temperature, then one had to run these massive gels, that were cumbersome and fragile at the same time. Next you had to dry the gel, without cracking it and expose it to X-ray film and then read the sequence. This was not as simple as it is today. Today things are much simpler all one needs to do is to carry out the PCR of the region of interest, phone the courier and send it to the sequencing service and in a day or two the sequence will be in the mailbox.

The question that set be off on this journey of disovery was simple: **What is the reason for the high HBeAg-negativity seen in southern African Black carriers of HBV?**

The most obvious place to begin was by sequencing the **precore/core region** that encodes for HBeAg. The precore/core region is transcribed into the precore mRNA that is translated into the precore/core fusion protein that is the precursor of the HBeAg. This precursor is modified by cleavage on either end to give the final product HBeAg that is secreted in the serum and expressed on the liver cells.

In the late 1980s it was noted that HBeAg-negativity in Greek and Italian patients was caused by a **G1896A** in the precore region. This mutation converts the TGG codon for amino acid tryptophan to a TAG, stop codon, leading to the truncation of the HBeAg precursor and loss of HBeAg expression. At the outset we were convinced that this was the reason for the high HBeAg-negativity in South Africa. However, G1896A was only found in a minority of isolates sequenced. What we know now, that we did not know then, was that the genotype A is the strain predominating in South Africa whereas genotype D prevails in the southern

Mediterranean and 1 nucleotide difference between the strains prevented the development of G1896A. Let me explain.

In order to be reverse transcribed the RNA intermediate of HBV, pgRNA has to be folded into a secondary structure known as the encapsidation signal or ε. The region that codes for this intermediate overlaps the region that encodes for HBeAg. In the strains circulating in Greece and Italy position 1858, which base pairs with 1896 is a T. In the case of genotype A strains, which circulate in South Africa 1858 is a C and it forms a stable base pair with the G at position 1896. This stable bond precludes a G to A mutation. On the other hand, the T-G, which is what we call a wobble pair and therefore not stable therefore the G1896A mutation is favoured in isolates with T at position 1858 and this is why we seen the development in genotype D and not A. One nucleotide can make a big difference!

Very little was known about the strains circulating in African populations. By sequencing part of the region that codes for the envelope proteins members of our team and Sheila Bowyer at the then National Institute for Virology, recognized, a unique segment of genotype A, which is now called subgenotype A1. Although not initially involved in the identification of subgenotype A1, my team and I were the first to sequence and analyze complete subgenotype A1 genomes from South Africa and to extend our studies by looking at subgenotype A1 found in other geographical regions. Subgenotype A1 is the prevalent subgenotype of A found **in Africa**, whereas subgenotype A2 predominates outside Africa.

Outside Africa, A1 is confined to areas where there has been a history of relatively recent migration from Africa, including the Indian subcontinent and Latin America . Moreover, the intragroup divergence within in Africa is higher than that found outside Africa telling us that A1 has been endemic in Africa for longer. Although it has been hypothesized that subgenotype A1 was dispersed from Africa as a result of the trans-oceanic slave trade in the 15th to 19th centuries, this has not been tested using modern phylogenetic analyses. In a recently published paper we showed that Africa was the most probable source of dispersal of subgenotype A1 globally and its dispersal to Asia and Latin America occurred at approximately similar time period. Our phylogeographic analysis and certain unexpected findings, such as the co-clustering of Somalian and Latin American strains and the dispersal of subgenotype A1 from India to Haiti, correlated well with historical evidence, intimating the impact of the trade and slave routes of the 9th to 19th centuries A.D. on the dispersal of subgenotype A1. The plausible sites of origin and migration routes of subgenotype A1 are depicted in this slide and these coincide with the routes depicted by history books. In its most notorious and highly developed form the slave trade was a triangular trade. Cheap manufactures, textiles and rum were traded to middlemen for slaves, who were then transported to the Americas, with the return trip to Europe supplying sugar, cotton and tobacco that were sold at huge profits. Close to 12 million people were taken out of Africa by European slave traders, with 10-20 percent dying before reaching the Americas. A visit to the Gold Coast in Ghana in 2010 sensitized me to the horrors of the slave trade. The way the slaves were housed and transported and the poor hygiene conditions were definitely conducive to the transmission of disease. Because subgenotype A1 is the predominant strain in

southern Africa a major objective of our research has been to characterize this unique subgenotype of A.

In order to do this we work in three broad areas of research that overlap.

- **Clinical and epidemiolgical characterization**, which involves recruitment of patients with and without disease and various diagnostic assays. Here we work very closely with clinicians, who look at symptoms and lab tests whereas as virologists we try to understand the virus and how is causes disease so we can develop interventions.
- **Molecular/ bioinformatics:** HBV DNA isolated from patients is sequenced and genotyped using bioinformatics *in silico* or what we call dry labwork. Essentially bioinformatics in the computational branch of molecular biology. When I first encountered phylogenetic trees they fascinated me and decided to get more training in this new discipline. I was fortunate to be able to attend two courses with world expert Anne-Mieke Van Damme in Ghent in 1999 and 2002. So in 2002 and 2004 we published one of the first phylogenetic analysis of full genomes of HBV from Africa. In 2007, I was awarded the National Bioinformatics Network Senior Fellowship in order to develop my bioinformatics skills further. As the number complete genomes increased we were able to compare the sequences of subgenotype A1 with A2 and other genotypes and to establish the distinguishing features of subgenotype A1. When our dataset was small this could be done manually. With the progress in sequencing technology the number of sequences we were generating as a team increased exponentially and it became necessary to develop tools that could automate the processes and can facilitate fast throughput of our sequencing analysis. Two of these have been published and are freely available on the internet and can be used for any sequencing data. This has been possible because we are fortunate to have a in house bioinformatician, who is trained in both molecular biology and computer science.

- **Functional characterization:** Once we have identified various mutations or variations in our sequences we characterize them using various model systems. This includes *in vitro* techniques using tissue culture and *in vivo* using animal models.

  I need to expand on this. When I worked with simian cytomegalovirus for my PhD I could infect bone marrow stromal cells by applying the virus to the cells in tissue culture. HBV can only infect primary hepatocytes, but the use of cells is limited by their variability and short life span. So as an alternative we used transfection of hepatoma cells with HBV DNA, which are cell lines derived for liver cancers as our *in vitro* model. Transfection is the introduction of HBV DNA directly into the liver cells. For this purpose replication competent plasmids have to be prepared. The HBV DNA is inserted into bacterial DNA, which acts as a vector or carrier that allows the HBV DNA to be accurately and efficiently copied. I show the strategy on this slide so that you can realize that this construction of replication competent clones is not a simple process. When these copies of the virus are then introduced into the hepatoma cells, where all the viral components can be expressed and the resulting virions are indistinguishable from the original

virion. In order to compare the replication of subgenotype A1 to the other strains circulating in South Africa we constructed replication clones of subgenotypes A1, A2 and D3.

The in vivo **urokinase plasminogen activator severe combined immunodeficient (uPA-SCID)** transgenic mouse model was utilised. This mouse has a defective immune system and a defective liver. Human liver cells are injected and these cells replace the defective mouse liver These cells can then be infected with HBV and we were able to demonstrate the successful infection of the uPA-SCID transgenic mouse model with subgenotype A1 of HBV.

Let me share some of our results with you.

Our clinical and epidemiological studies carried out in liver disease patients and asymptomatic carriers of the virus from South Africa, India, Hong Kong and Japan have shown that individuals infected with subgenotype A1 have the following features:

- Lower HBV DNA levels: this was signicantly lower both at the HBeAg positive and negative stage of infection.
- Lose HBeAg in the serum much sooner. This was highly significant in individuals younger than thirty years.
- Higher levels of liver damage as measure by a marker that measure programmed cell death or apoptosis.
- Higher incidence of HCC By genotyping HBV isolated from 111 HCC patients and 111 ASCs, we showed a 4.5-fold increased risk for HCC [95% confidence interval: 1.86-10.90] and an earlier age at cancer diagnosis (on average, 6.5 years younger) in carriers infected with subgenotype A1 compared with genotype D.

This characteristics were demonstrated in our functional studies. HBeAg expression was lower in cells transfected with A1 compared to A2 and D. In the uPA-SCID mouse A1 expressed lower levels of HBV DNA, HBcAg and HBeAg.

The next step was determine whether there are distinct characteristics of subgenotype A1 that may contribute to these clinical characteristics. Extensive bioinformatics analyses revealed that the gene for HBeAg has a number of distinctive mutations/variations compared to subgenotype A2 and D and which could account for the high HBeAg-negativity and liver damage. These include the following mutations A1762T/G1762A GCACKozakTCAT, G1862T and G1888A.

Although A1762T/G1762A is not only found in subgenotype A1 and we did not find an direct association of these mutations with HBeAg-negativity when HBeAg-positive sera with the mutant virus were compared with sera with wild-type virus, HBeAg-titres were reduced by 60% in the patients carrying the mutant virus ($p<0.0001$). Moreover, 66% of HCC patients had the mutations compared to only 11% of the asymptomatic carriers.

We found the subgenotype A1 isolates harbour double or triple mutations in the Kozak sequence, immediately upstream of the precore AUG codon, which might impair HBeAg expression, as a result of sub-optimal translational initiation. This hypothesis was tested, using site-directed mutagenesis and transfection experiments that showed that HBeAg expression was impaired by the Kozak mutations, by a leaky scanning mechanism. The effect of the double mutations on HBeAg expression was comparable with that of the common core promoter mutations (1762T1764A) and independent of HBx expression . Furthermore, the presence of 1762T1764A, together with 1809-1812 mutations, reduces HBeAg expression in an additive manner. At the post-translational level, a G to T transversion at 1862 of the precore region could conceivably affect HBeAg expression by interfering with signal peptide cleavage.

So instead of the single G1896A mutation which truncates the HBeAg precursor we have shown that subgenotype A1 utilized alternative mechanisms to modulate HBeAg expression: at the transcriptional, translational and post-translational levels. Moreover, recently completed longitudinal study we have demonatrated that both the Kozak TCAT in the BCP and G1862T can evolve and not necessarily simulatneously. GCAC occurred with 1862G and the conversion of GCAC to TCAT in the Kozak pre-empted the substitution G1862C, which occurred before 1862T.

At the post-translational level, a G to T transversion at 1862 of the precore region could conceivably affect HBeAg expression by interfering with signal peptide cleavage. The reason for this is that the phenylalanine introduced by this mutation is a “forbidden” amino acid at position -3 and its aromatic ring sterically hinders the activity of the signal peptidase, leading to a decreased efficiency of signal peptide cleavage. This leads to decreased expression of HBeAg and its accumulation in the endoplasmic reticulum, leading to proteotoxicity and leading to apoptosis. This was shown both in tissue culture using HBeAg-expressing plasmids and in the animal model.

HBeAg has common epitopes with HBcAg and has been postulated to act as an immunotolerogen. Reduced levels or absence of HBeAg from the serum, as a result of infection with subgenotype A1 isolates with mutations or substitutions that affect HBeAg expression, can lead to HBcAg being targeted by both the cellular and humoral immune reponses. This, together with mutations such as G1862T can lead to necrosis of hepatocytes and liver damage, which is an important contributing factor in the development of HBV-associated HCC. We have also shown that cells transfected with subgenotype A1 have a high accumulation of replicative intermediates which are known to promote the integration of HBV DNA in chromosomal DNA. Our studies have shown multiple integrants in liver tumours from patients infected with subgenotype A1 the development of deletion mutants in the S region of HBV isolated from HCC patients. Confounding factors include environmental factors such as alcohol intake, iron overload and aflatoxin and co-infection with other viruses.

No infectious disease studies in Africa can neglect the HIV pandemic scourging our continent. Of the 34 million adults and children living with HIV globally, 22.5 million reside in sub-Saharan Africa. In order to look at HBV/HIV co-infection we determined the prevalence and characteristics of HBV infection in anti-retroviral naïve HIV-positive adults entering a rural cohort in Mpumalanga province. 24% of 300 HIV-infected adults were HBV DNA positive. Of these 9% were HBsAg-positive whereas the remaining 15% were HBsAg-negative. This study provided important information on the molecular characteristics of HBV in HIV-infected southern Africans prior to ART initiation these include:

- 97% were infected with subgenotype A1.
- 33% of HBV isolated from HBsAg-negative individuals had G1862T
- S region deletion mutants previously found in isolates from HCC patients were detected
- 10% of the isolates had lamivudine resistance mutations L129M, V173L and L180M.

These findings have important clinical relevance in the management of HBV/HIV coinfection. Because the majority of infections were HBsAg-negative, HBV infection will remain undetected serologically and thus nucleic acid testing, which can detect HBV DNA in the presence or absence of HBsAg in the serum, needs to be implemented. Deletion mutants, previously shown to occur in HBV from HCC patients, were detected. These could be a risk factor for the development of HCC in HIV patients, whose lifespan is being increased and immune system reconstituted following the introduction of ART. Furthermore, the presence of mutants resistant to LAM, prior to the initiation of LAM-containing ART, has important implications and repercussions, and highlights the need for the inclusion in the treatment regimen of tenofovir (TDF), to which these mutants are sensitive.

So in summary what we have here is a virus that replicates at low levels, loses its HBeAg expression very early, so that by the time a woman is at the childbearing age she is HBeAg-negative, which means that mother to child transmission is rare at birth and this strain of virus kills its host at a very early age by being hepatocarcinogenic. Moreover, this virus has a high sequence diversity, which tells us that it has been endemic in the populations it infects for a very long time. How has it survived in the population? In a study carried out with the South African National Blood Transfusion service we have shown that the virus is transmissible horizontally at very low levels of 32 (22-43) HBV DNA copies per 20 ml of plasma at a rate of 0.34 per million. Furthermore, the only risk factor of HBV infection in HIV infected patients was the number of lifetime sexual partners, implicating sexual transmission of HBV. Although the exact means of horizontal transmission has not been elucidated, environmental, behavioural and cultural factors play a role. Cultural practices like scarification and tattooing practices, use of sharp objects, significantly increase the risk of HBV infection. Iatrogenic procedures including tonsillectomy and indiscriminate injections or the re-use or use of non-sterilized syringes have also been shown to increase the risk of HBV infection.

In the last 10 years we have seen considerable progess in HBV genotyping research in Africa. The shaded areas represent large scale studies whereas the dots represent were only a few isolates were genotoyped. The studies marked with a green asterisk were carried out by our team. However, we can remain complacent for four reasons:

- Firstly Africa is unique. There are 65 million HBV carriers in Africa, about a quarter of the world's chronic HBV carriers and this is probably an underestimate because of lack of surveillance and underreporting. We have one of the highest incidences of HCC as a result of HBV infection as well as the heaviest burden of HIV infection. There are still regions where we have no information about the genotypes circulating. This includes Mozambique, with high HCC incidences in the world, Botswana with high HIV burden and Ethiopia a land of poverty.
- Secondly, as I hope I have clearly demonstrated the strains circulating in Africa differ from those found outside Africa and have unique characteristics and I have not even spoken about genotype E, which has displaced genotype A in western and central Africa. Very little is known about the natural history of infection with genotype E. Our and other studies have shown that in contrast to subgenotype A1, individuals infected with genotype E have a high frequency of HBV positivity and high viral loads.
- Thirdly, genotypes previously considered to be uniquely African are being isolated outside Africa as a result of emigration and modernd travel. This means that we are seeing the export of HBV from a continent where the virus is hyperendemic to low endemicity areas of the world. The introduction of the virus to these regions, where universal vaccination is not generally practiced, will definitely hamper efforts for the global eradication of HBV.
- Finally, studies of African isolates may assist in revealing the origin of HBV and to clarify the many questions about its evolution.

Research is not a solitary exercise and good collaborations are essential for good science. I have been privileged to have had outstanding mentors who have inspired, taught and guided me. I have supervised enthusiastic postgraduate students and postdoctoral fellows, with whom we have been able to move the research field forward. I have been honoured to collaborate with world experts in HBV genotype research and to have been involved in this field from its early development to its maturation. Finally, I want to thank my current team for their support and pay tribute to my immediate family Costa, Irini and Kimon, who have made the greatest sacrifices so that I can be standing here tonight.