

HIGH INCIDENCE OF A HEPATITIS B VIRUS PRES2 DELETION IN WEST AFRICA AMONG HBV CHRONIC CARRIERS : ASSOCIATION WITH HEPATOCELLULARCARCINOMA

Prevention Of Liver Fibrosis and Cancer in Africa

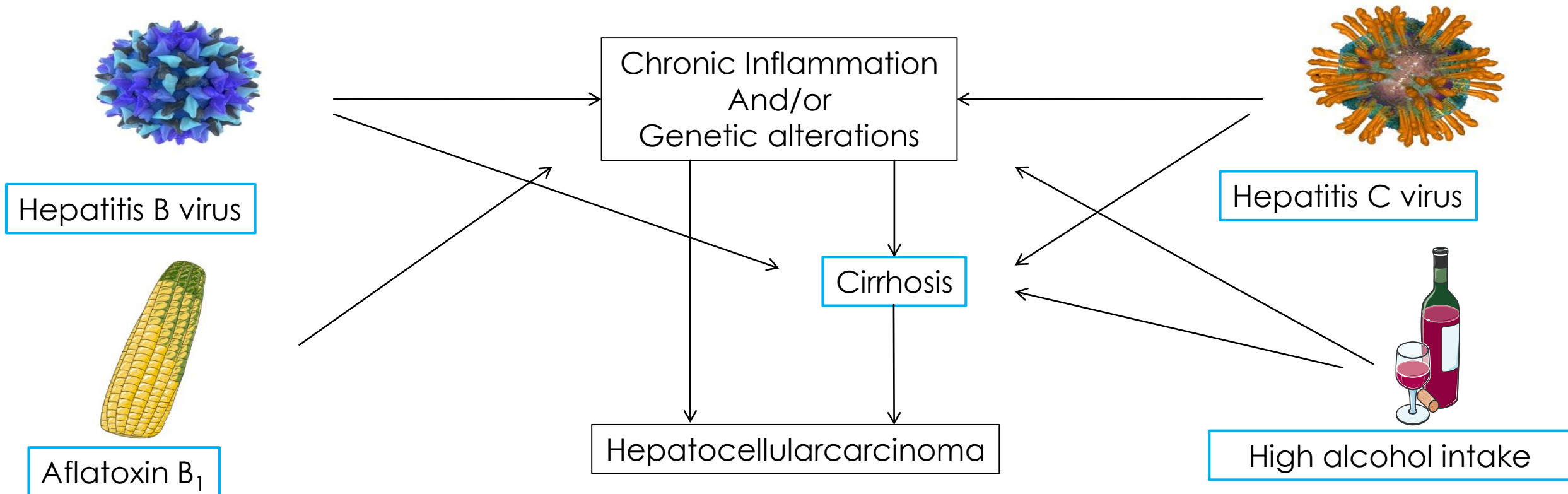


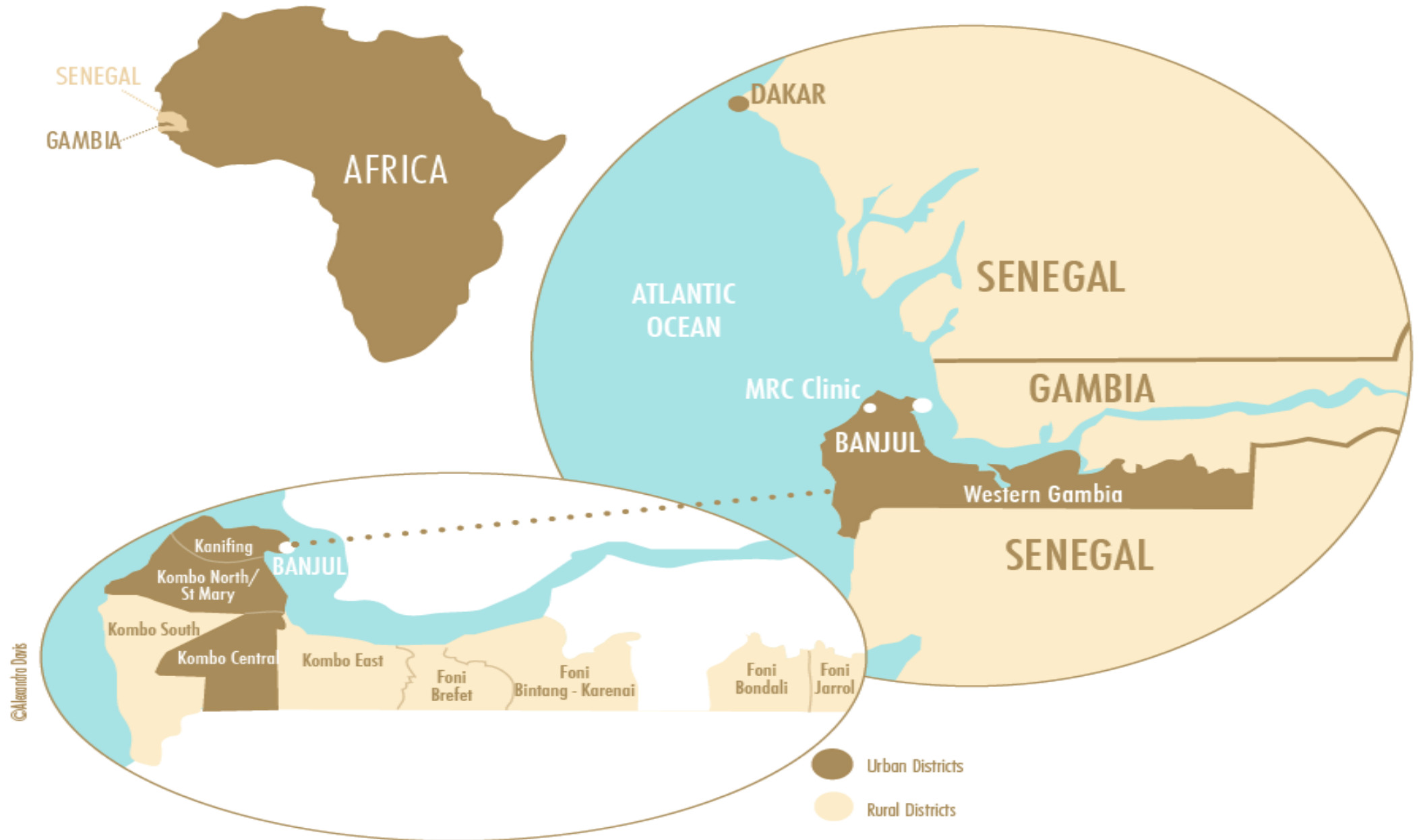
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Hepatocellular carcinoma

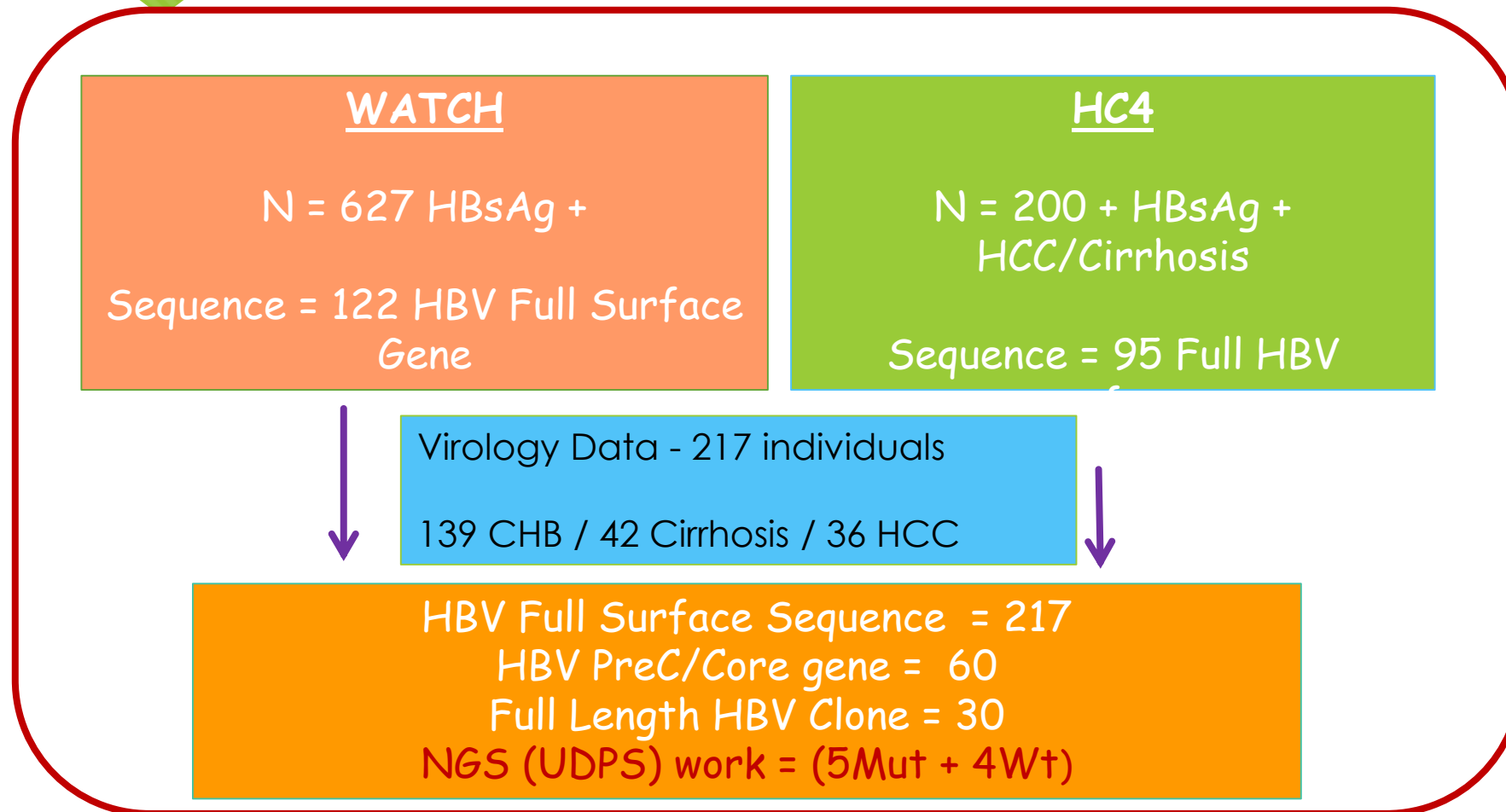
Risk factors





Map of The Western Gambia indicating the urban and rural community areas

PROLIFICA Cohort / Samples

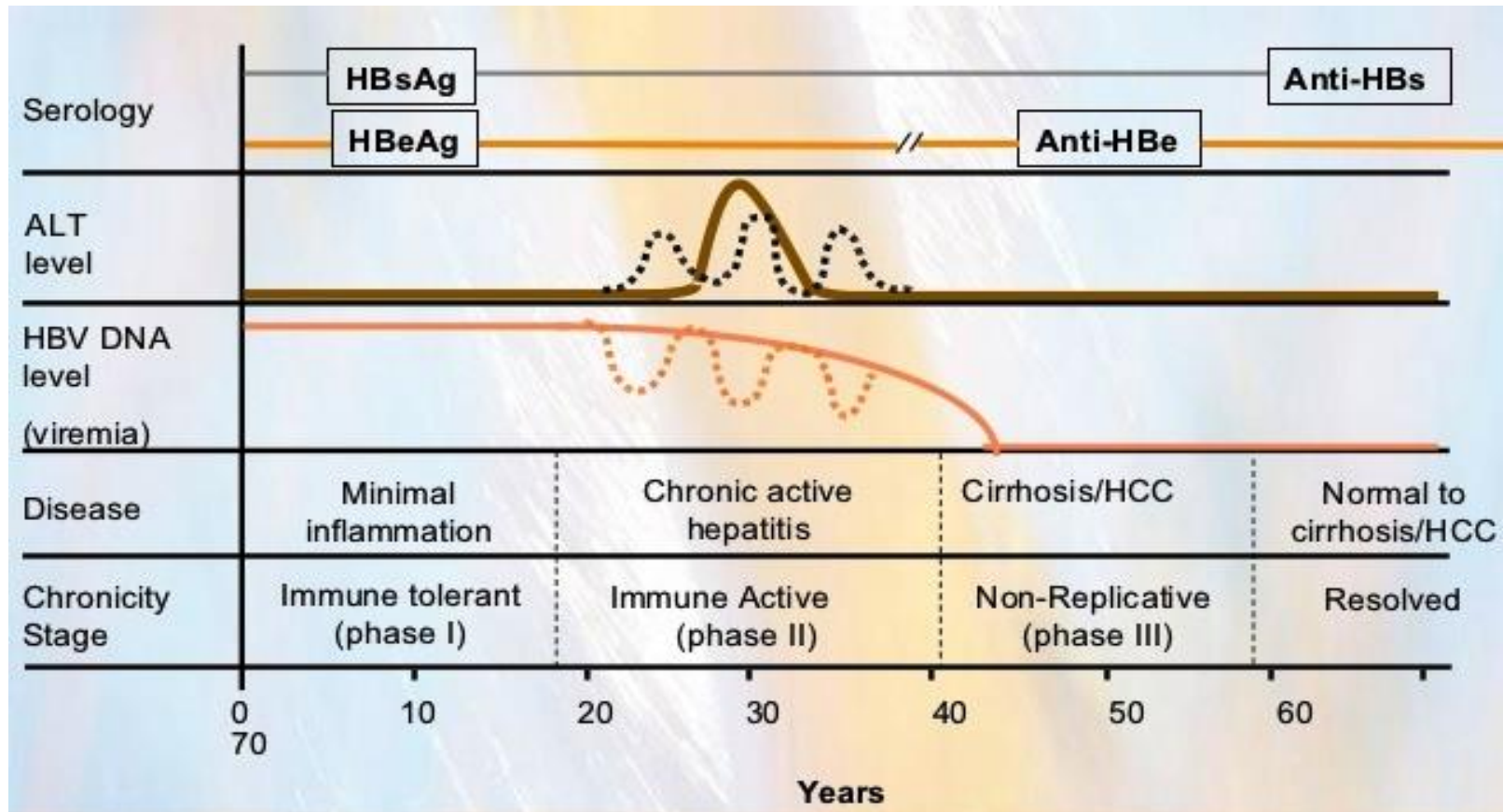
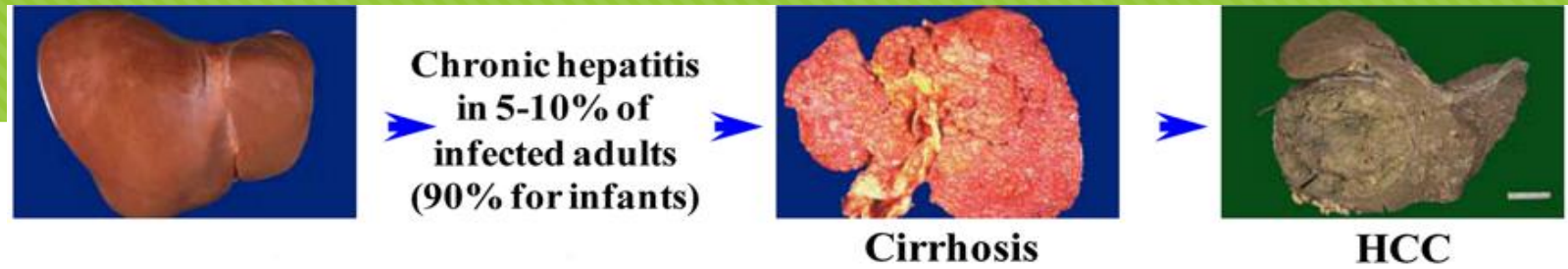


HBV infection : virological factors for HCC development

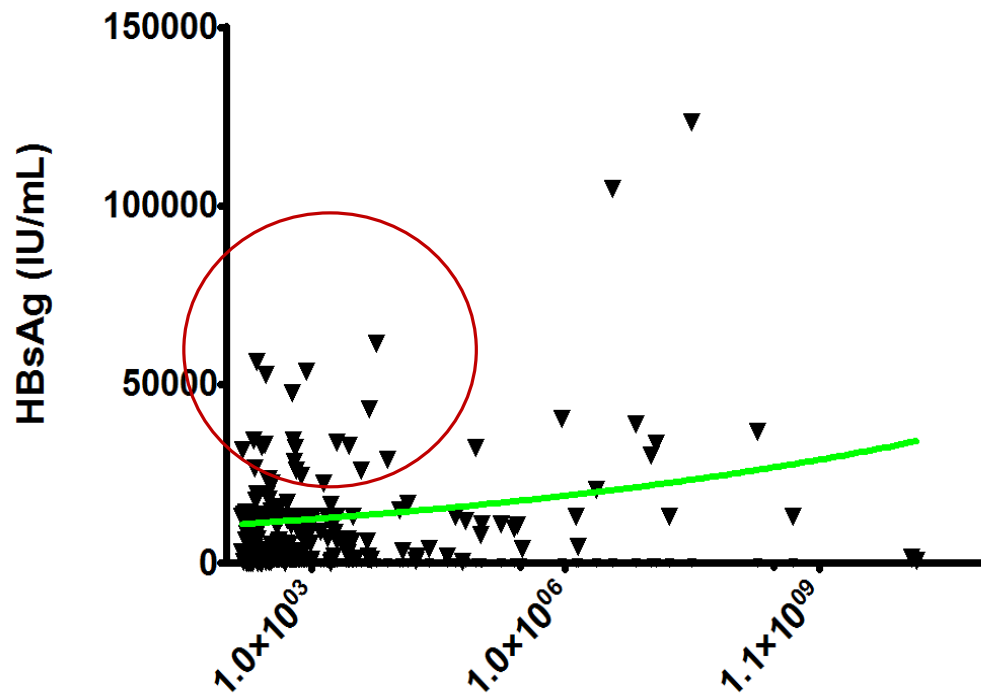
Within the Watch and HCC case-control study platform we did:

- Explore contribution of HBV genotype and variants in the development of HCC.
- Explore HBV serological markers and viral loads
- Generate genome sequences of circulating strains.
- Identify viral genetic patterns and investigate their association with liver disease.

Natural History of HBV infection and HCC development



Demography and Epidemiological profile



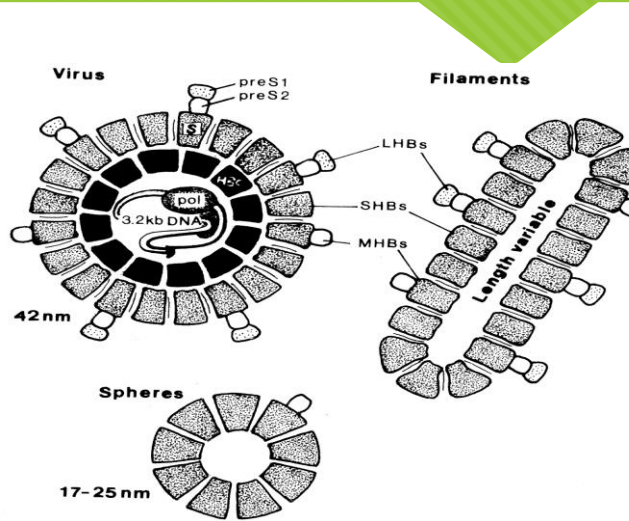
□ Mostly infected with **Genotype E** (>75%) and rest Genotype A, few E+A recombinant

□ >95% are **HBeAg negative** infection and sero-converted.

□ Very **low viral load** (<500 IU/ml) some with **High HBsAg**

HBsAg quantification Vs HBV Viral load

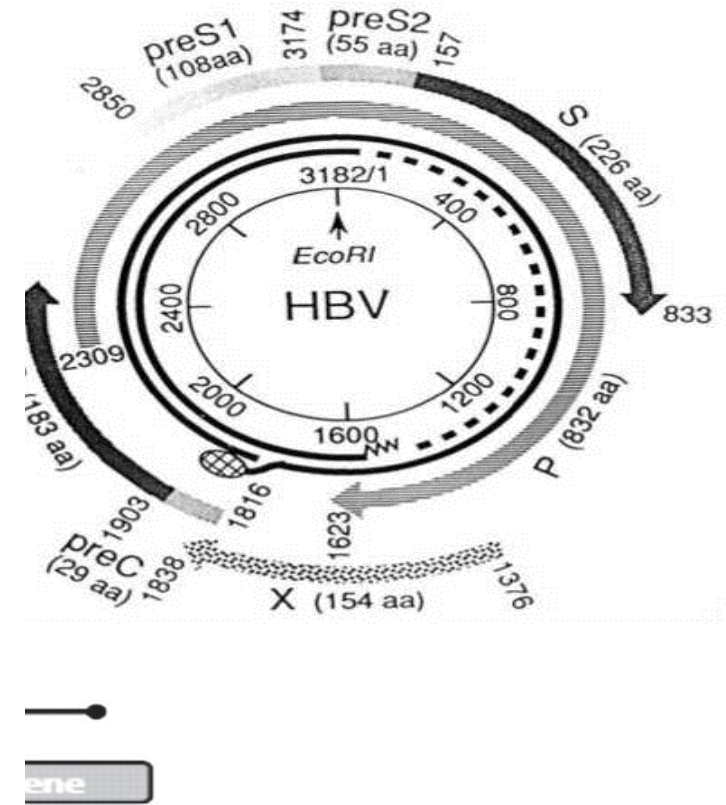
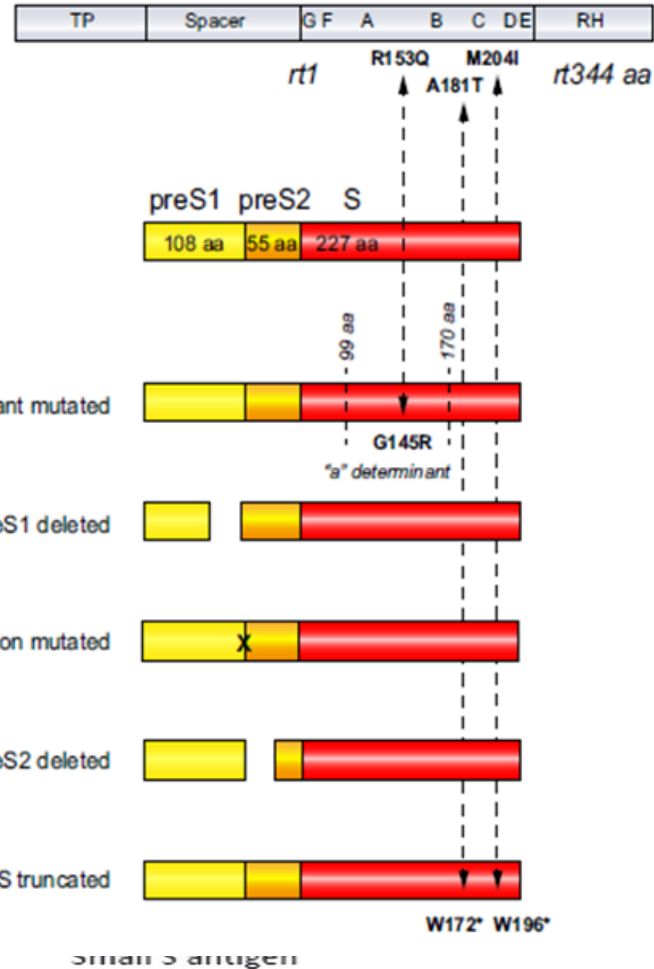
Hepatitis B Virus (HBV)



Polymerase gene

Wild type preS/S gene

preS/S gene mutants



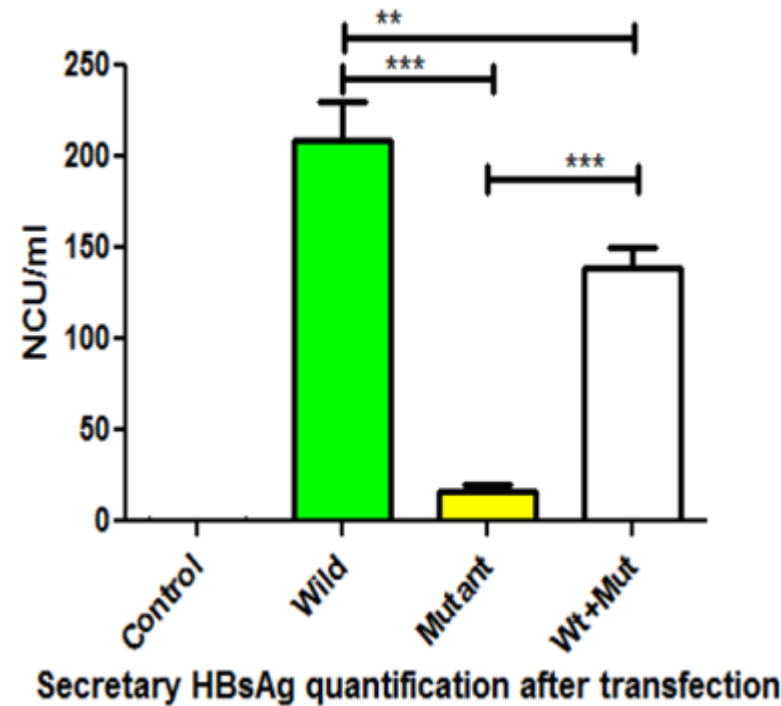
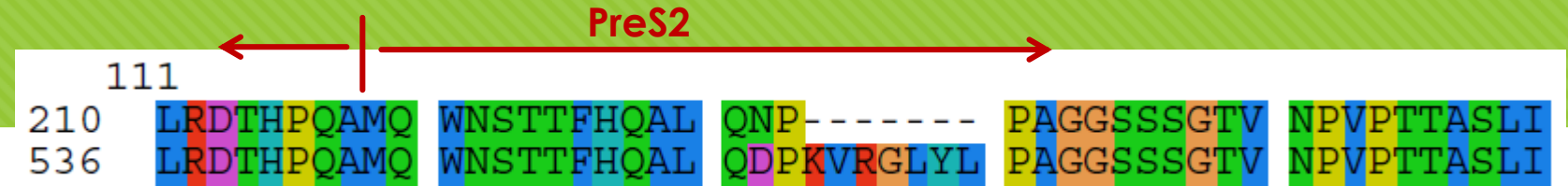
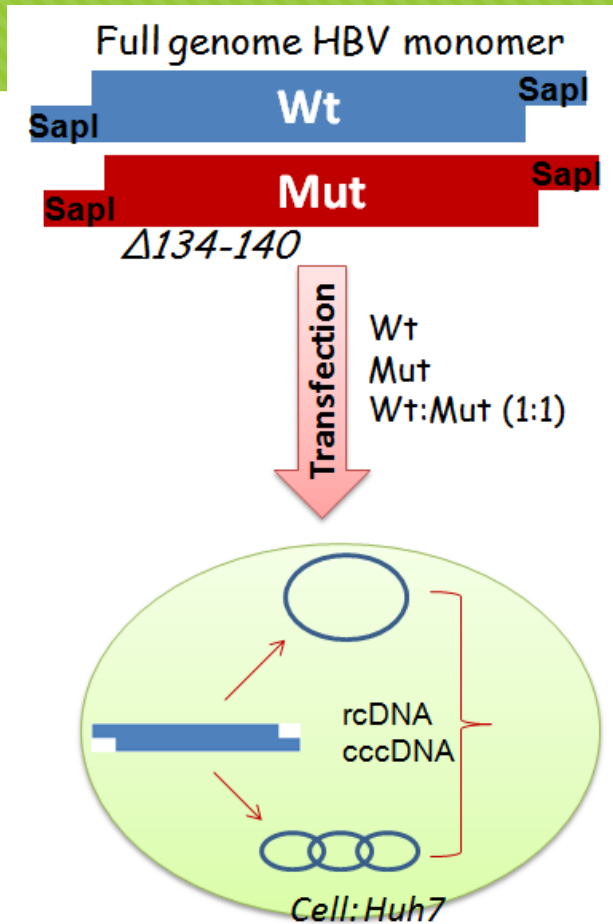
Pollicino et al., 2014 J Hep.

Demographic, Biochemical and HBV profile

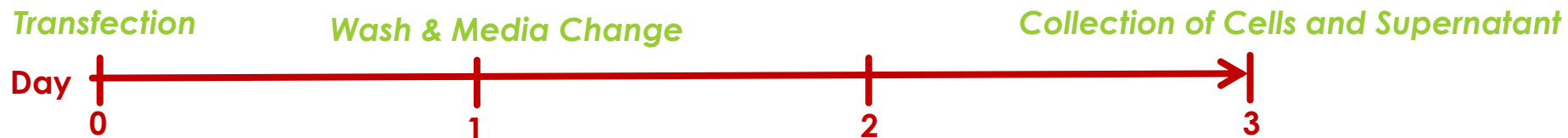
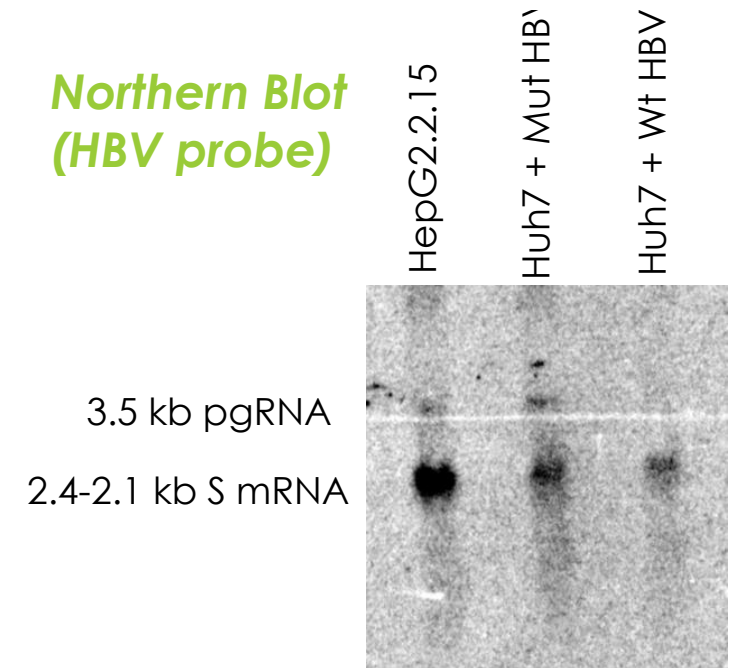
	No LC/HCC	LC	HCC	p-value [#]	Total
N (%)	139 (64%)	42 (19.35%)	36 (16.58%)		217 (100%)
Age, Yr (Mean±SD)	28.1 ± 40.8	37.9 ± 15.8	41.3 ± 13.6	p = 0.05	39.2 ± 12.9
Sex (Male : Female); (% Male) [§]	82 : 57; (59%)	31 : 11; (74%)	28 : 8; (77%)	<i>p1</i> = 0.102, p2 = 0.050 , <i>p3</i> = 0.793	141 : 76; (65%)
ALT, IU/l (Mean±SD)	38.0 ± 57.2	77.3 ± 199.7	118.0 ± 167.1	<i>p</i> = 0.001	58.6 ± 122.8
AST, IU/l (Mean±SD)	40.0 ± 36.8	121.6 ± 273	393.2 ± 222.1	<i>p</i> = 0.0001	108.4 ± 193
AFP, ng/ml (Mean±SD)	7.5 ± 14.6	28.1 ± 40.8	5147 ± 8427	<i>p</i> = 0.0001	885.2 ± 3939
HBV DNA, IU/ml, (Mean±SD)	1.1E+8 ± 1.9E+9	1.7E+7 ± 7.9E+7	4.6E+6 ± 1.2E+7	<i>p</i> = 0.896	8E+7 ± 1E+9
HBeAg Negative (N, %)	125 (89.9%)	32 (76.1%)	25 (69.4%)	p1 = 0.035 , p2 = 0.005 , <i>p3</i> = 0.610	182 (83.8%)
Anti-HBeAg Seroconversion (N, %)	111 (88.8%)	30 (93.7%)	25 (100%)	<i>p1</i> = 0.5, <i>p2</i> = 0.127, <i>p3</i> = 0.498	166 (91.2%)
HBsAg Quantitative IU/ml, (Mean±SD), n	19319 ± 26382, 133	6907 ± 6743, 26	7048 ± 13407, 19	p = 0.01	16106 ± 23896
HBV Genotypes					
Gen E (N, %)	125 (90%)	36 (86%)	22 (61%)	<i>p1</i> = 0.414, p2 = 0.001 , p3 = 0.018	183 (84.3%)
Gen A (N, %)	14 (10%)	6 (14%)	14 (39%)		34 (15.6%)
PreS2 Deletion (N, %)	34 (24%)	16 (38%)	20 (55%)	<i>p1</i> = 0.1141, p2 = 0.0005 , <i>p3</i> = 0.1719	75 (32.2%)

[#] (*p* = ANOVA test *p* value; *p1* or *p2* or *p3* = Fisher's exact test *p* value of no LC/HCC vs LC or noLC/HCC vs HCC or LC vs HCC respectively); [§] (% of male sex was used for Fisher's exact test)

In-vitro Characterization

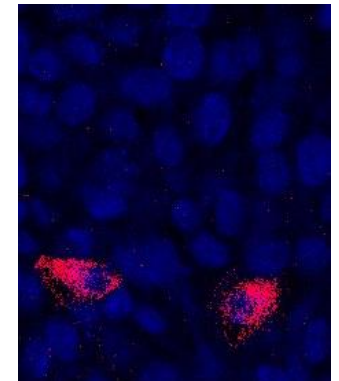
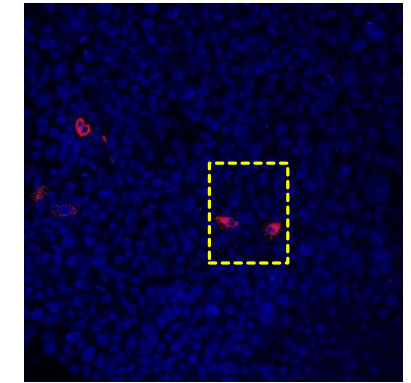
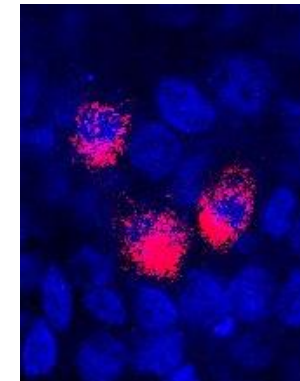
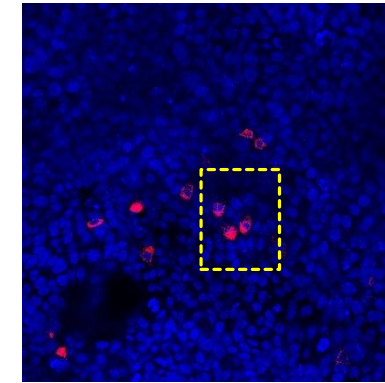
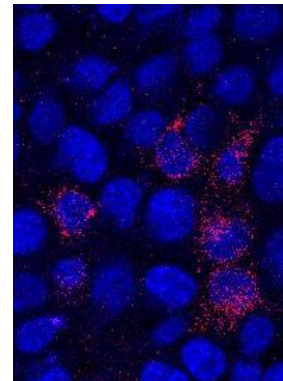
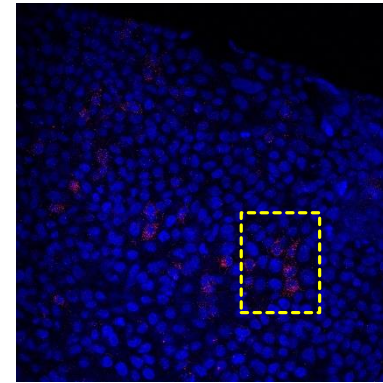
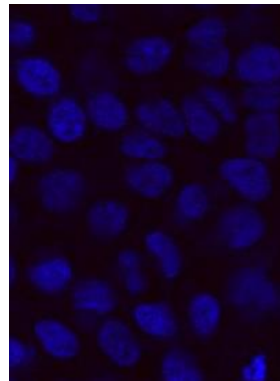
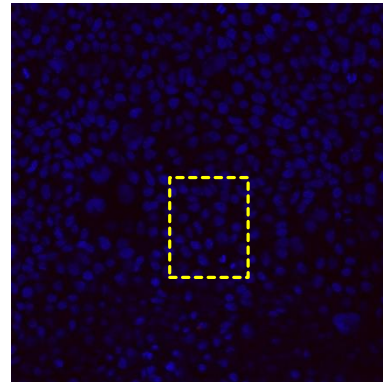


Northern Blot
(HBV probe)



Immune-Fluorescence on transfected Huh7 cells (in-vitro localization)

Stained with **DAPI (blue)**
and **anti-HBs (red)**
monoclonal and
visualized in confocal
microscope



HBV Wt (Δ PreS2)
HBV Mut (Δ PreS2)

-

+

-

+

-

-

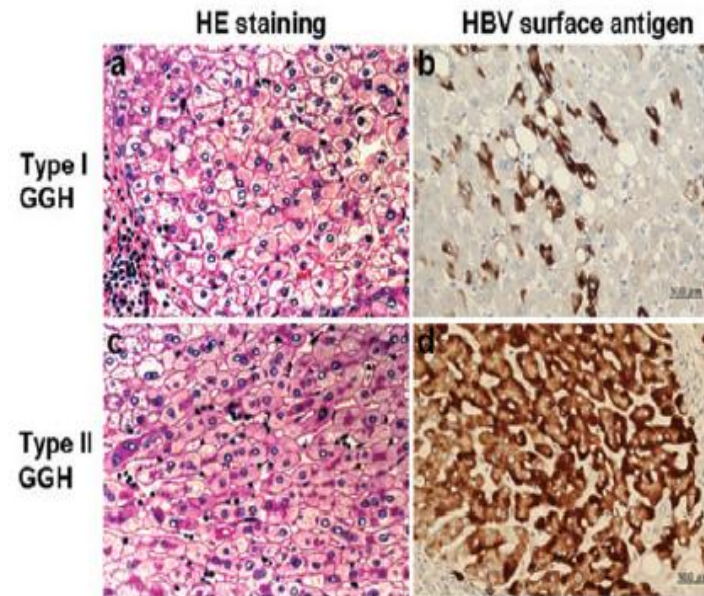
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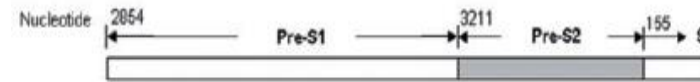
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Ground Glass Hepatocytes (GGH) and PreS deletion

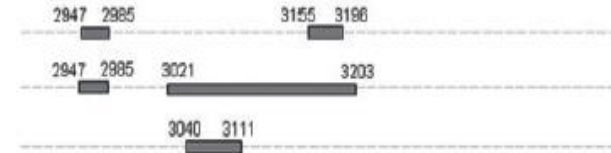
Su et al., 2008 JGH



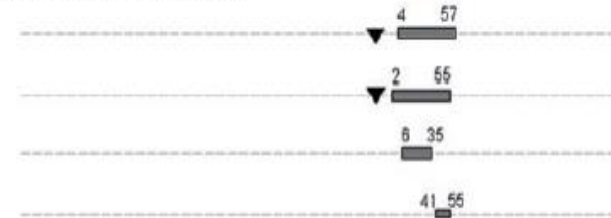
Wild type



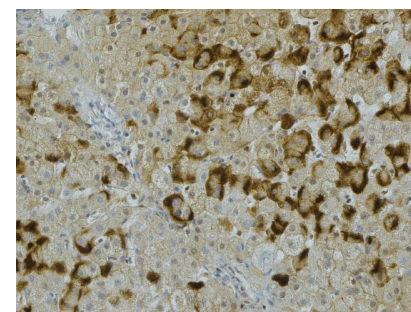
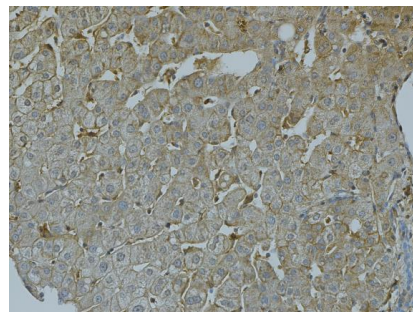
Pre-S1 deletions



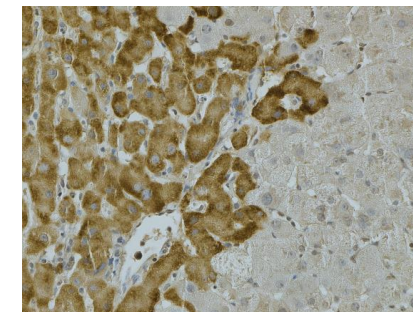
Pre-S2 deletions ▼ ATG→ATA



Prolifera Samples
IHC



GGH, Type I

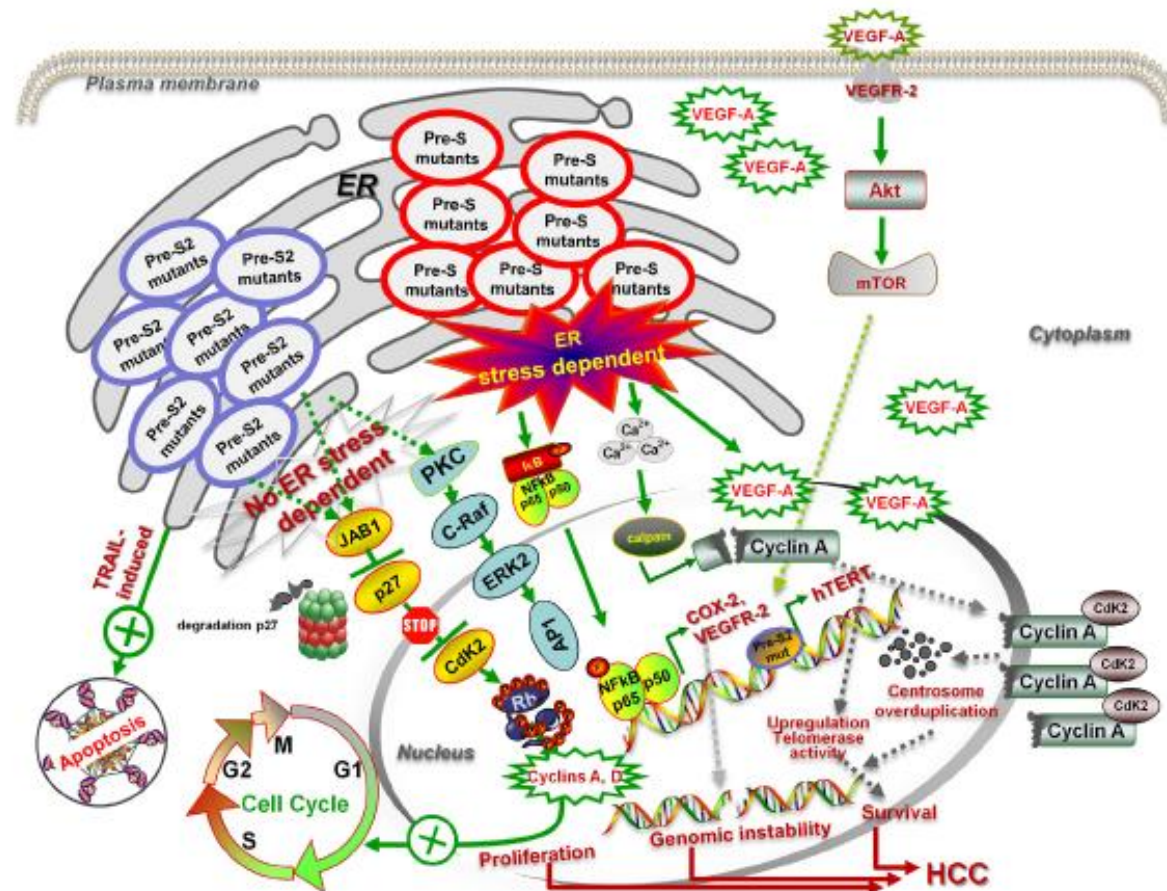


GGH, Type II

Models for HCC associated with HBV PreS Mutants

Pollicino et al., 2014

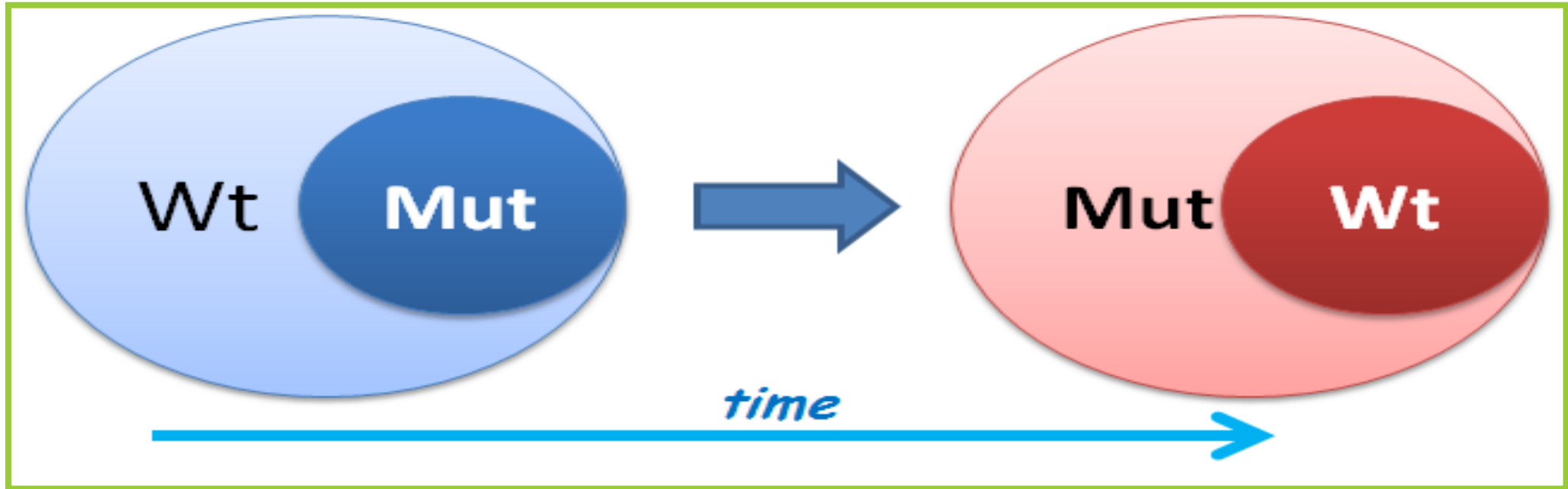
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□ PCR screen

□ IHC and confocal microscopy

Natural History of HBV infections in West Africa



Frequency of those Pre S2 deletion mutant: Early onset of HCC despite low viral loads due to ER stress

Mutants may interfere with DNA damage repair pathways: to be explored and exploited to open treatment strategies?

Acknowledgements...



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London

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HBeAg negative strains are often mutant for BCP/CP

BCP or CP Mutation	HBeAg Negative				HBeAg Positive
	Total, n=47 (%)	CHB, n=19 (%)	LC, n=11 (%)	HCC, n=17 (%)	Total, n=13 (%)
A1762T	28 (59%)	9 (47%)	7 (63%)	12 (70%)	7 (53%)
G1764A	28 (59%)	9 (47%)	7 (63%)	12 (70%)	7 (53%)
G1896A	37 (78%)	14 (73%)	9 (81%)	14 (82%)	0
G1899A	21 (47%)	6 (31%)	5 (45%)	10 (58%)	0
A1762T/G1764A/G1896A/G1899A	44 (93%)	19 (100%)	11 (100%)	14 (82%)	7 (53%)
A1762T + G1764A	26 (55%)	8 (42%)	7 (63%)	11 (64%)	7 (53%)
A1762T + G1764A + G1896A	21 (44%)	6 (31%)	6 (54%)	9 (52%)	0
A1762T + G1764A + G1896A + G1899A	8 (17%)	2 (10%)	1 (9%)	5 (29%)	0

BCP/CP mutation is very high (80-90%), may be one of the reason for high incidence of HBeAg negative infection (90 - 95%)