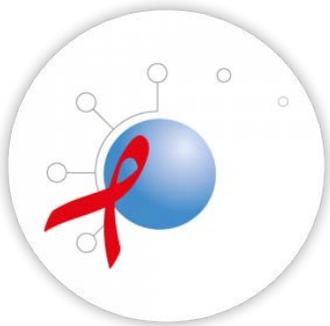


Genetic elements clustered in specific immune active HBsAg regions drive HBV reactivation under immunosuppression: an extensive analysis of HBV genome

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Treatment Strategies & Antiviral Drug Resistance**

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HBV research moves forward —receptors and reactivation

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- The **increasing usage of several classes of immune-suppressive drugs**, revolutionizing the treatment of onco-haematological and immunological diseases, has highlighted **the relevance of HBV reactivation in a wide spectrum of medical fields**
- This is leading to an **uprising interest in HBV reactivation** that is characterizing the field of HBV research

Background

- HBV reactivation is defined as an **abrupt increase of serum HBV-DNA** in patients with **chronic as well as resolved HBV infection** (*Hwang and Lok, 2014*), undergoing an immune-suppressive therapy.
- HBV reactivation can cause **severe liver injury** resulting in jaundice, liver failure, and even death.
- Single-case reports have suggested a **higher degree of HBsAg genetic diversity** in patients experiencing HBV reactivation, supporting the role of HBV genetic variability in this phenomenon.
- However, **molecular mechanisms and viral characteristics underlying immunosuppression-driven HBV reactivation** have not been clarified yet.

Objective

To provide a map of genetic markers along HBV genome correlated with HBV reactivation driven by immune-suppressive therapy

Methods

This study included
127 patients, **all infected with D genotype HBV:**



47 Patients with HBV-reactivation driven by immunosuppressive therapy

80 chronically infected patients, naïve to antiviral drugs used as control

- The key inclusion criteria for HBV-reactivation population were: Patients >18 years with documented HBV reactivation driven by immunosuppressive therapy (including anti-cancer drugs and corticosteroids).
- Chronically infected control patients had no clinical evidence of cirrhosis/hepatocellular carcinoma
- HIV, HCV or HDV coinfections were exclusion criteria for both populations

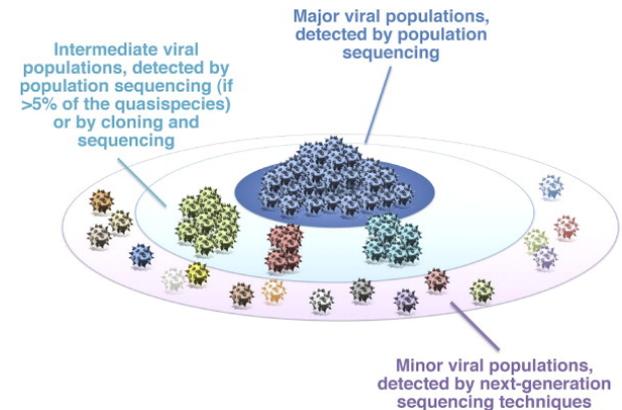
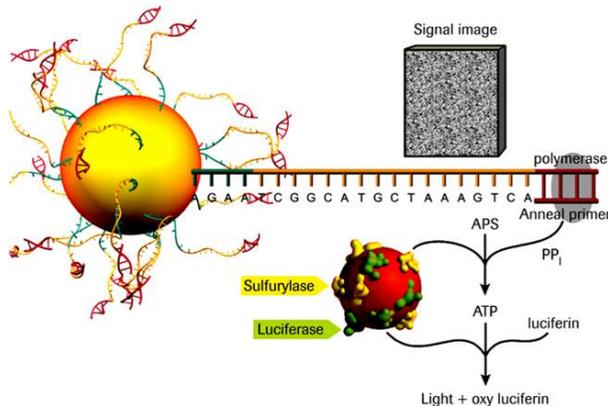
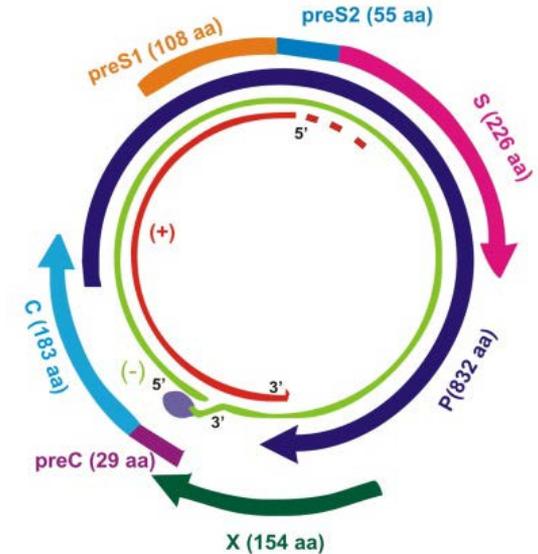
Methods

- The genes coding for HBV RT, HBsAg and HBcAg were sequenced by using a population based approach (Genetic Analyzer ABI 3130XL)

- The mean genetic distance of the different HBV genes was estimated as the extent of nucleotide substitutions per site determined by the Tajima-Nei model (MEGA)

- Association of mutations with HBV reactivation was assessed by Fisher exact test.

- For 30 HBV-reactivating patients and 24 control patients ultra-deep sequencing (UDPS) of HBsAg was performed by using Roche 454 Junior



By UDPS, viral variants that are present with an inpatient prevalence < 20% can be detected

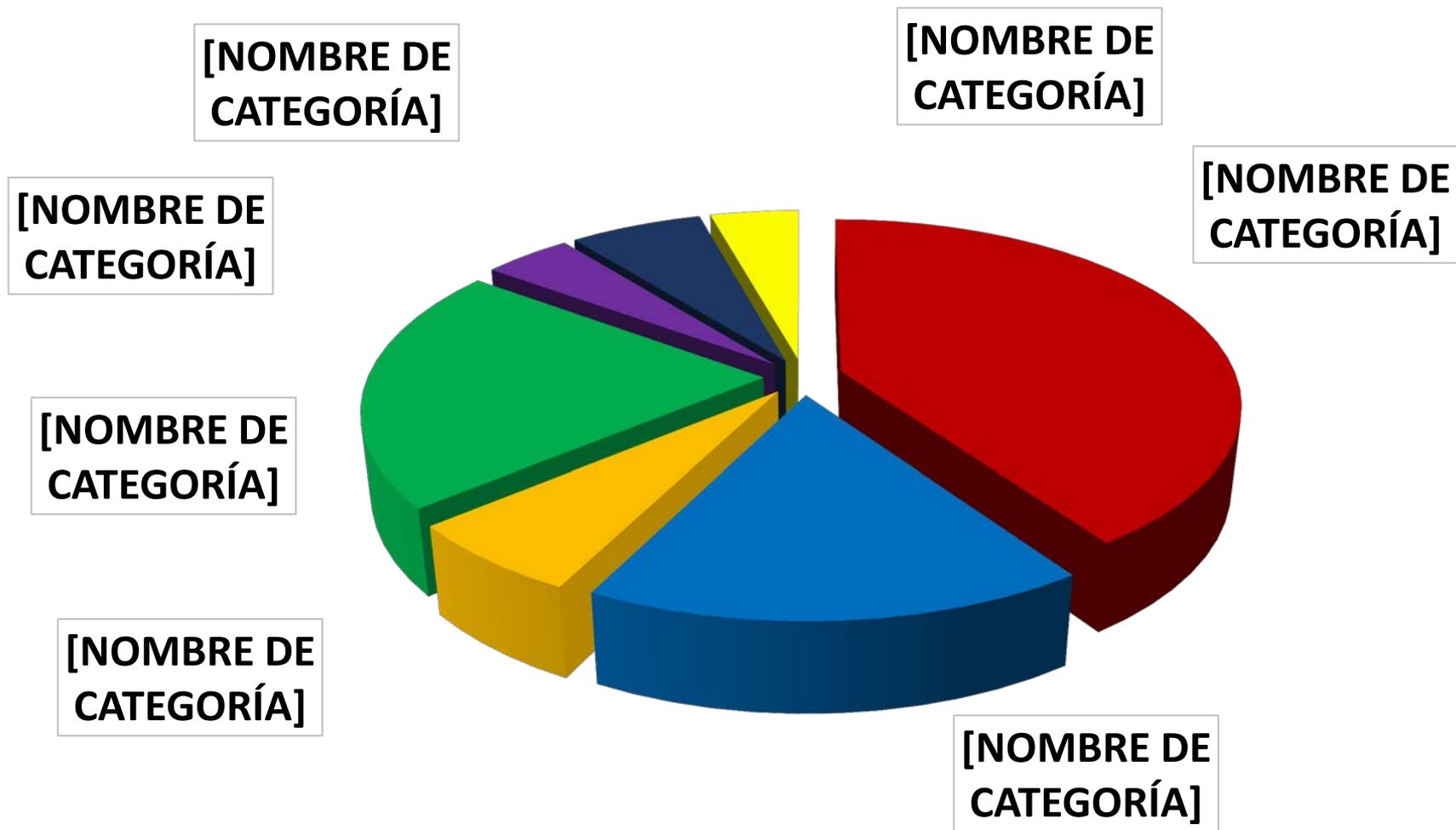
Results

Patients' Characteristics

Characteristics	HBV reactivating patients (N _{tot} = 47)	Controls (N _{tot} =80)
Male, N(%)	32 (68.1)	50 (62.5)
Median (IQR) Age [Years]	63 (58-72)	48 (38-63)
Median (IQR) HBV-DNA [log IU/ml]	5.9 (4.6-7.6)	4.7 (3.4-6.3)
Median (IQR) ALT [IU/L]	193 (48-568)	47 (29-100)
Median (IQR) AST [IU/L]	114 (35-251)	39 (23-72)

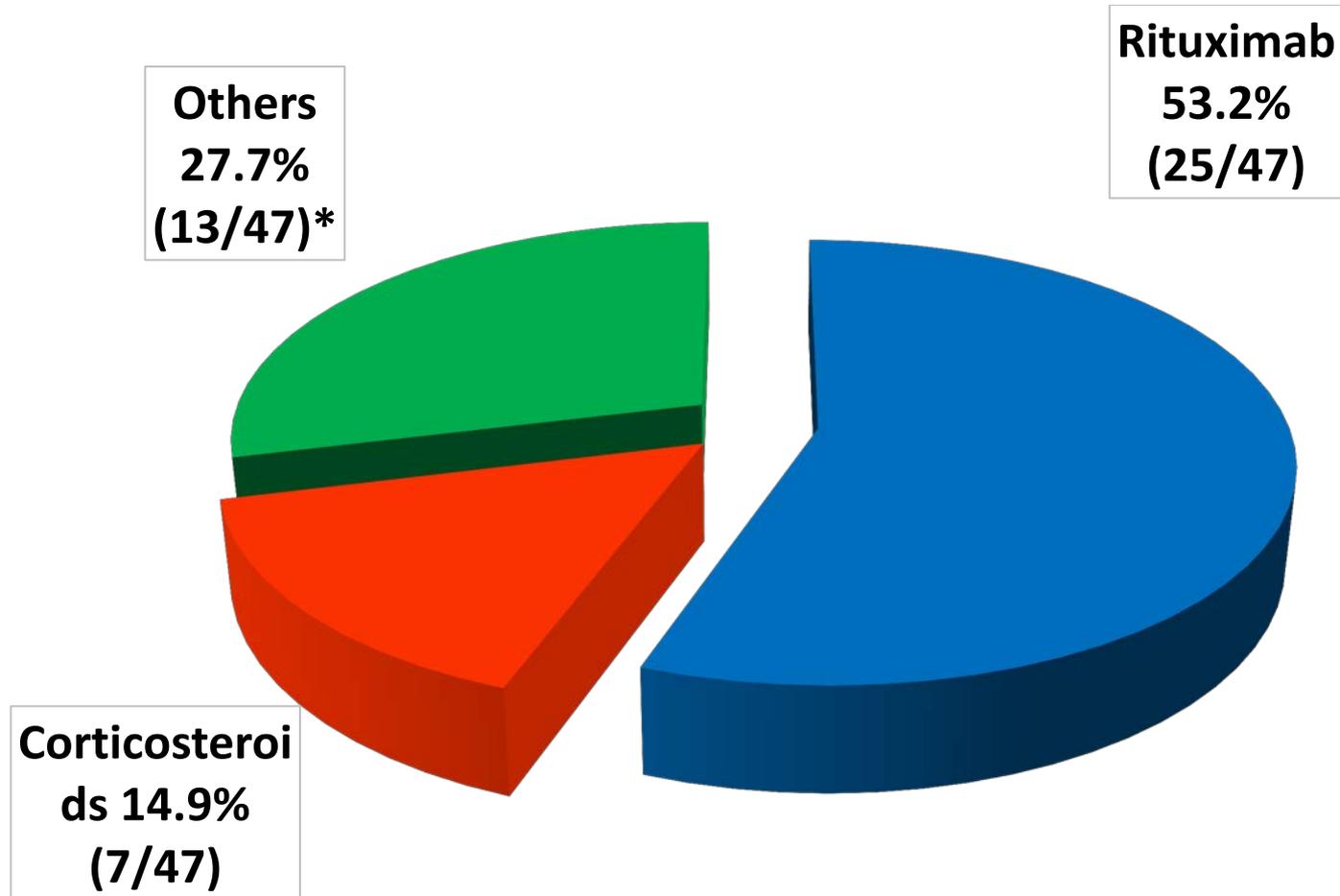
As expected, **HBV reactivated patients** were characterized by **high levels of HBV-DNA and increased transaminases** at the diagnosis of HBV reactivation

Status of HBV infection before reactivation: HBV reactivation occurs in a large variety of HBV serological profiles



The graph reports the status of HBV infection before reactivation according to the serological patterns of patients and level of serum HBV-DNA.

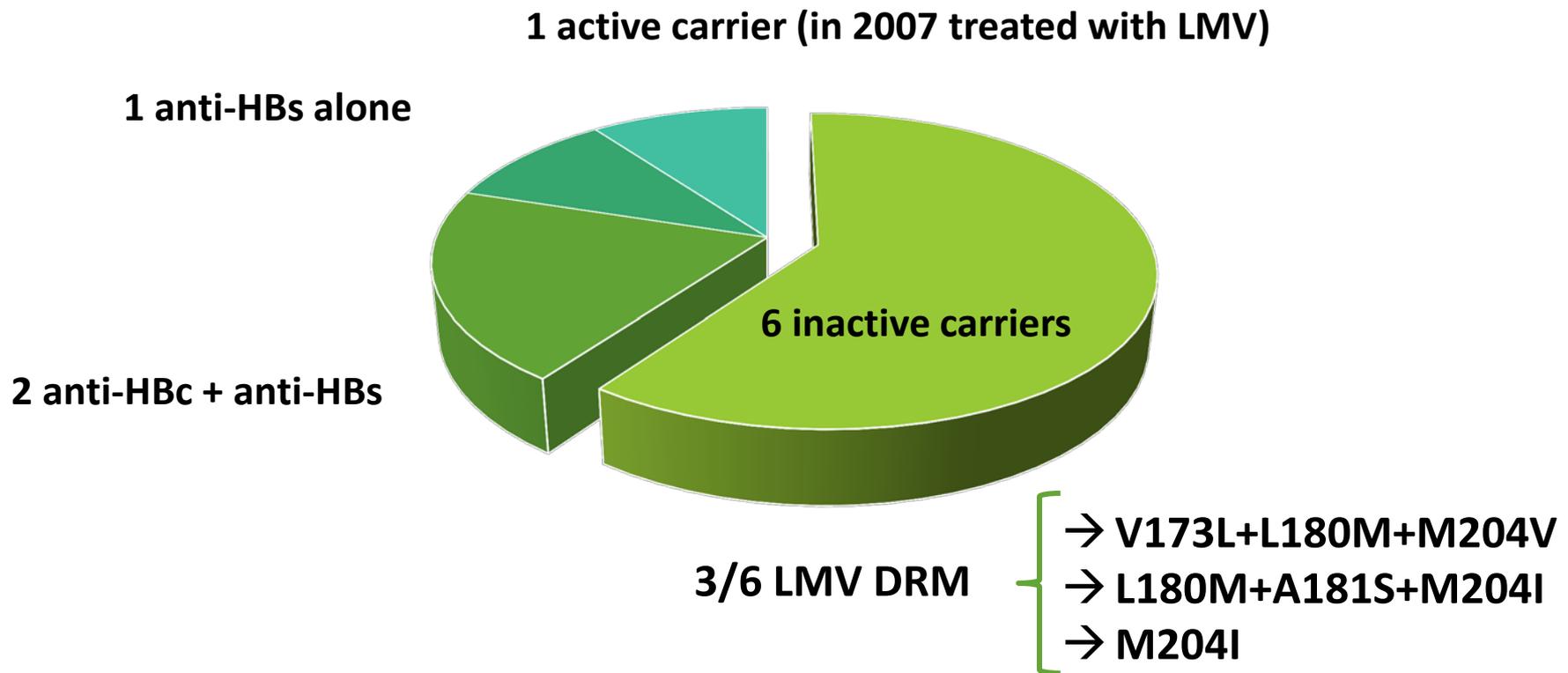
Types of immune-suppressive agents in our group of HBV reactivating patients



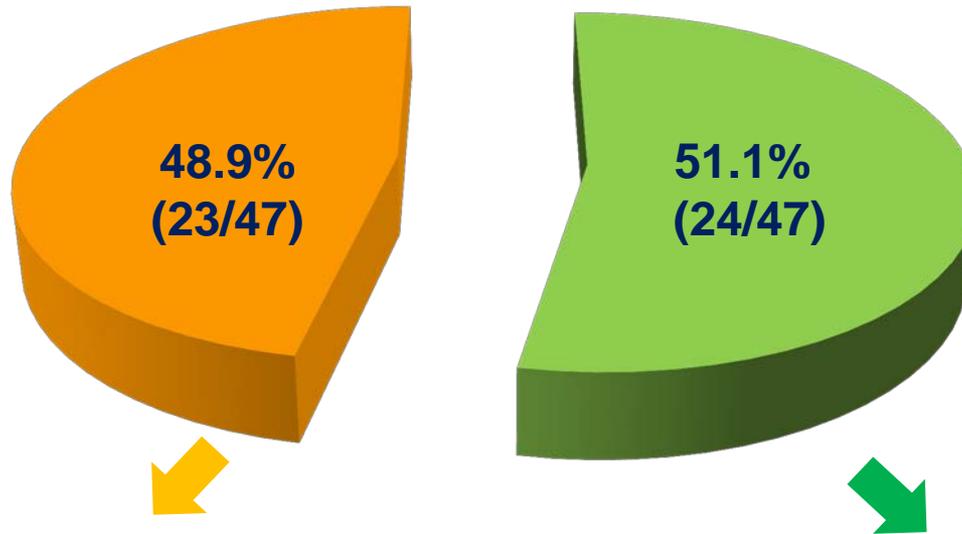
* fludarabine, everolimus+mycophenolate, methotrexate, vincristine+dexamethasone

10 patients experienced HBV-reactivation despite LMV treatment

Among them, **3 inactive carriers developed resistance** after 15, 25 and 60 months of LMV prophylaxis



More than half of patients developed HBV reactivation after completing immunosuppressive therapy



HBV reactivation during immunosuppression

HBV reactivation after completing immunosuppressive therapy

51.1% of patients experienced HBV-reactivation after completing immunosuppressive-therapy (**min-max: 1-48 months**)

HBsAg genetic variability is significantly higher in HBV-reactivating patients than controls. This increased variability is particularly focused within **major hydrophilic region**, target of neutralizing antibodies

Such increase is not observed in pre-S1/S2, RT...

HBV genomic region	Mean \pm SE genetic distance^a in:		P value
	HBV-reactivating pts(N=47)	Controls (N=80)	
PreS1	0.025 \pm 0.009	0.032 \pm 0.010	n.s.
PreS2	0.032 \pm 0.015	0.032 \pm 0.015	n.s.
HBsAg 226 aa	0.025 \pm 0.006	0.018 \pm 0.005	<0.001
N-terminal domain (aa 1-98)	0.012 \pm 0.006	0.009 \pm 0.005	0.179
Major hydrophylic region (aa 99-169)	0.053 \pm 0.016	0.041 \pm 0.014	<0.001
a-determinant (aa 110-140)	0.089 \pm 0.034	0.077 \pm 0.031	0.038
C-terminal domain (aa 170-226)	0.013 \pm 0.006	0.008 \pm 0.005	0.173
RT	0.041 \pm 0.007	0.040 \pm 0.006	0.98

^a The mean genetic distance of the different HBV genes sequences was estimated as the extent of nucleotide substitutions per site determined by the Tajima-Nei model of MEGA v5, applying a gamma distribution with shape parameter = 1.0. The estimate variance was assessed from the bootstrap method with shape parameter = 1,000, and the test was conducted for nucleotide alignment.

...and not even in **BCP/PreCore/Core**...indeed in this region HBV-reactivating patients are characterized by a **degree of genetic variability significantly lower than controls** ($P < 0.001$)

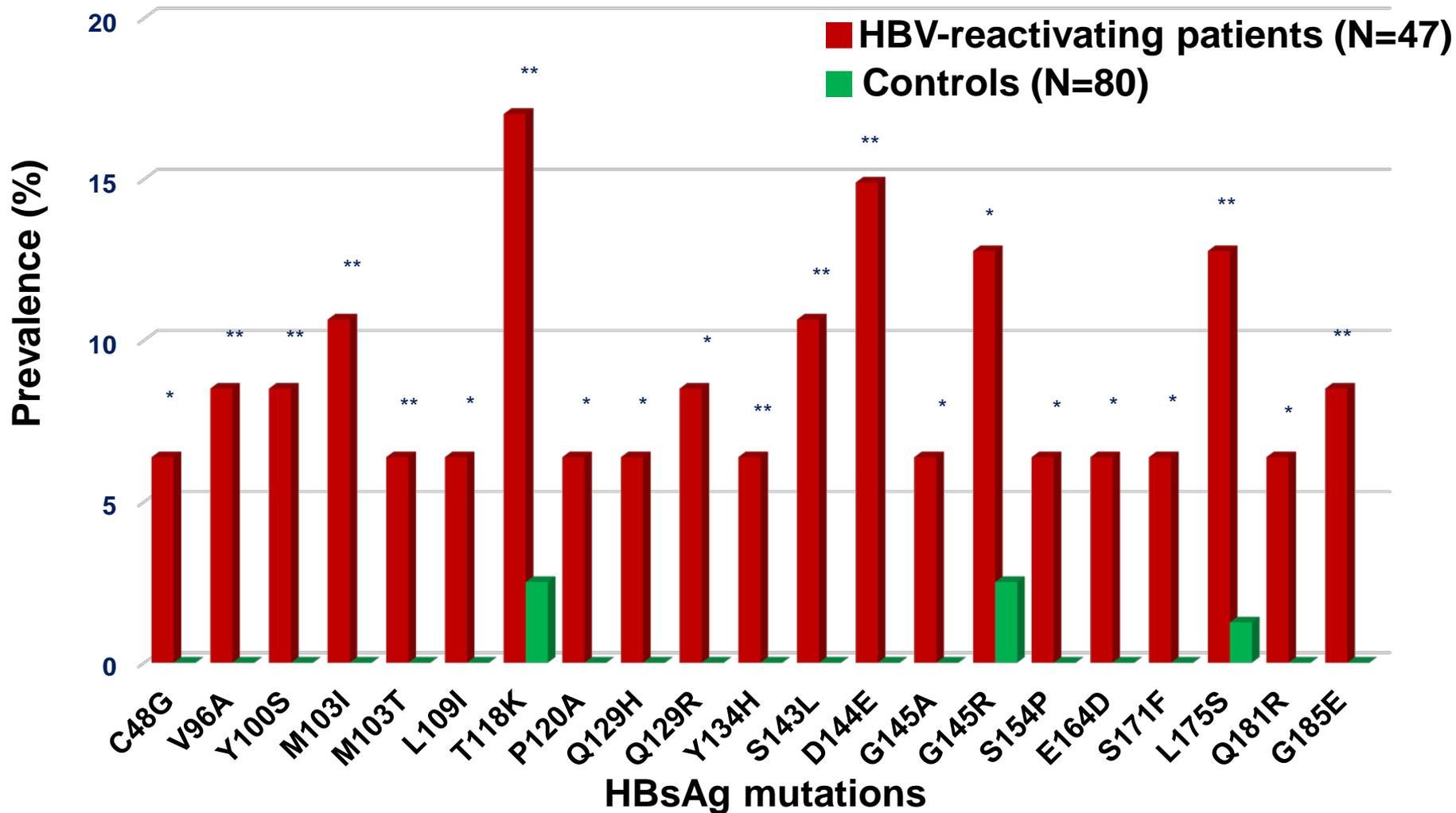
HBV genomic region	Mean \pm SE genetic distance**					
	HBeAg_neg		pvalue	HBeAg_pos		pvalue
	HBV-R pts (N=18)	Controls (N=58)		HBV-R pts (N=10)	Controls (N=8)	
BCP/preCore/Core	0.043 \pm 0.008	0.056 \pm 0.009	0.014	0.010 \pm 0.007	0.060 \pm 0.032	0.001
BCP	0.043 \pm 0.025	0.046 \pm 0.026	0.478	0.035 \pm 0.023	0.041 \pm 0.024	0.696
PreCore	0.015 \pm 0.012	0.017 \pm 0.014	0.534	0.004 \pm 0.004	0.023 \pm 0.015	0.009
Core	0.049 \pm 0.010	0.064 \pm 0.011	0.007	0.037 \pm 0.008	0.069 \pm 0.012	0.001

^a The mean genetic distance of the different HBV genes sequences was estimated as the extent of nucleotide substitutions per site determined by the Tajima-Nei model of MEGA5, applying a gamma distribution with shape parameter = 1.0. The estimate variance was assessed from the bootstrap method with shape parameter = 1,000, and the test was conducted for nucleotide alignment.

** HBeAg negative and positive patients were analyzed individually, in order to avoid confounding effects given by HBeAg status of the patients

These data strongly suggest that **HBsAg variability**, specifically clustered in a **region dense of immunogenic epitopes (MHR)**, gives a **specific contribution to HBV reactivation**

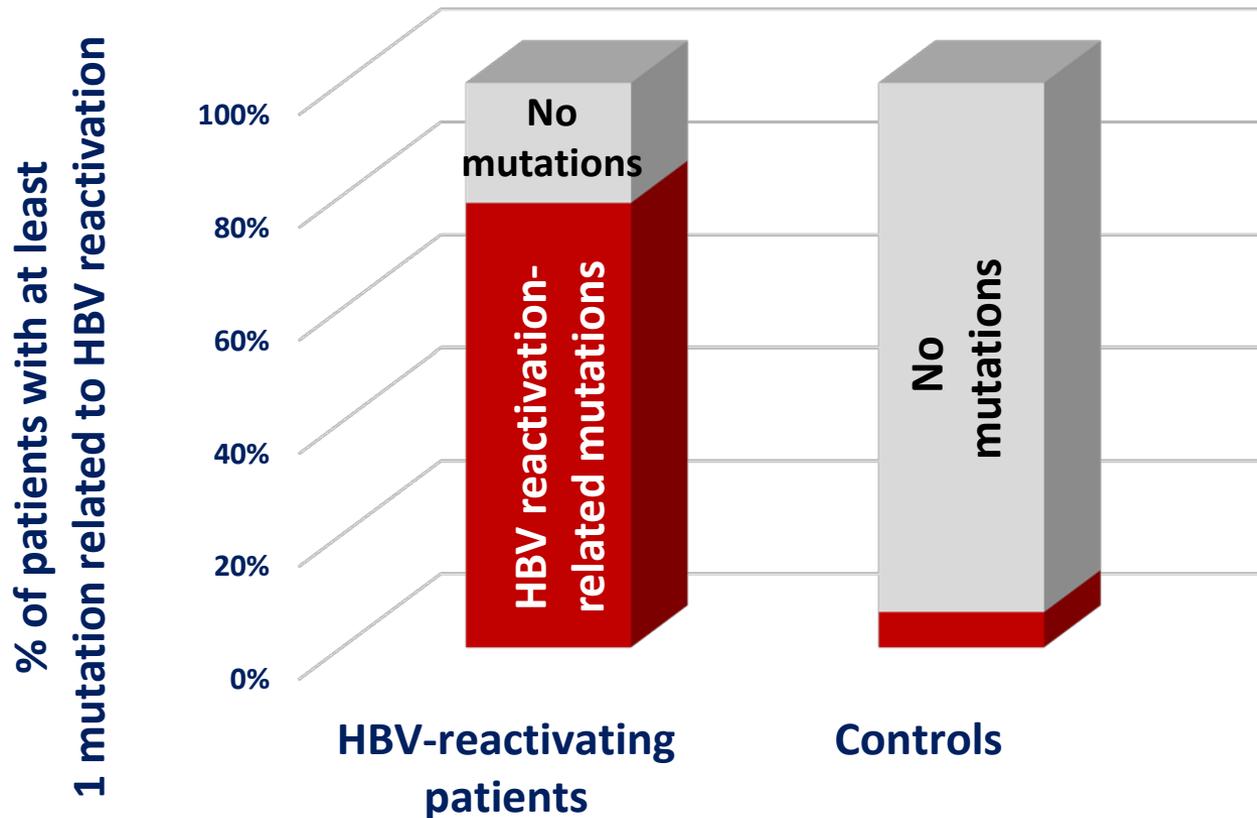
Specific HBsAg mutations significantly correlate with HBV reactivation



The histogram reports HBsAg mutations significantly correlated with HBV reactivation. HBsAg sequences were obtained by population-sequencing. Statistically significant differences were assessed by Fisher Exact Test.

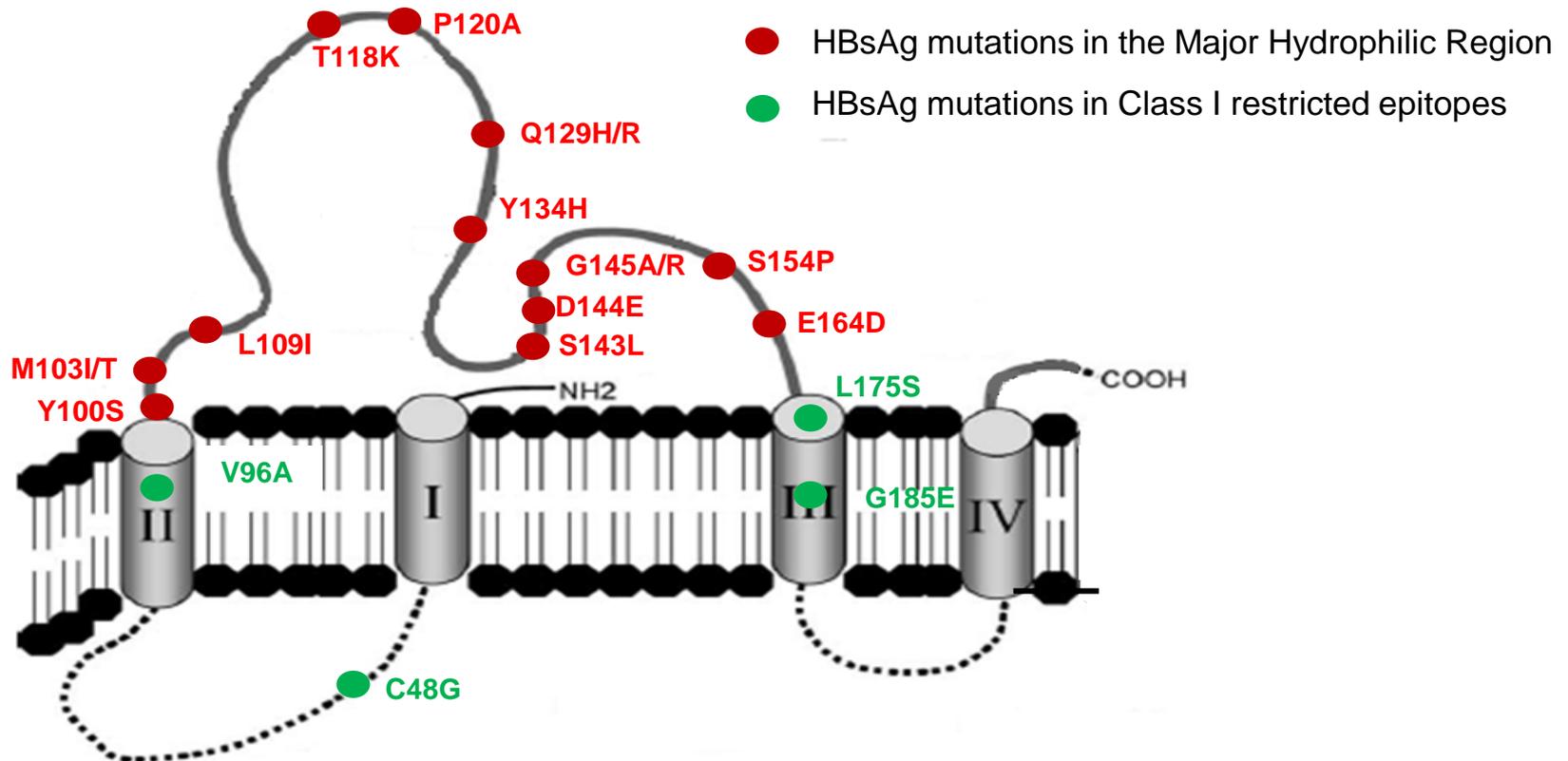
* P < 0.05. ** P < 0.01

78.7% of HBV-reactivating patients versus 6.2% of controls carries at least 1 of these HBsAg mutations related to HBV reactivation (P<0.001)

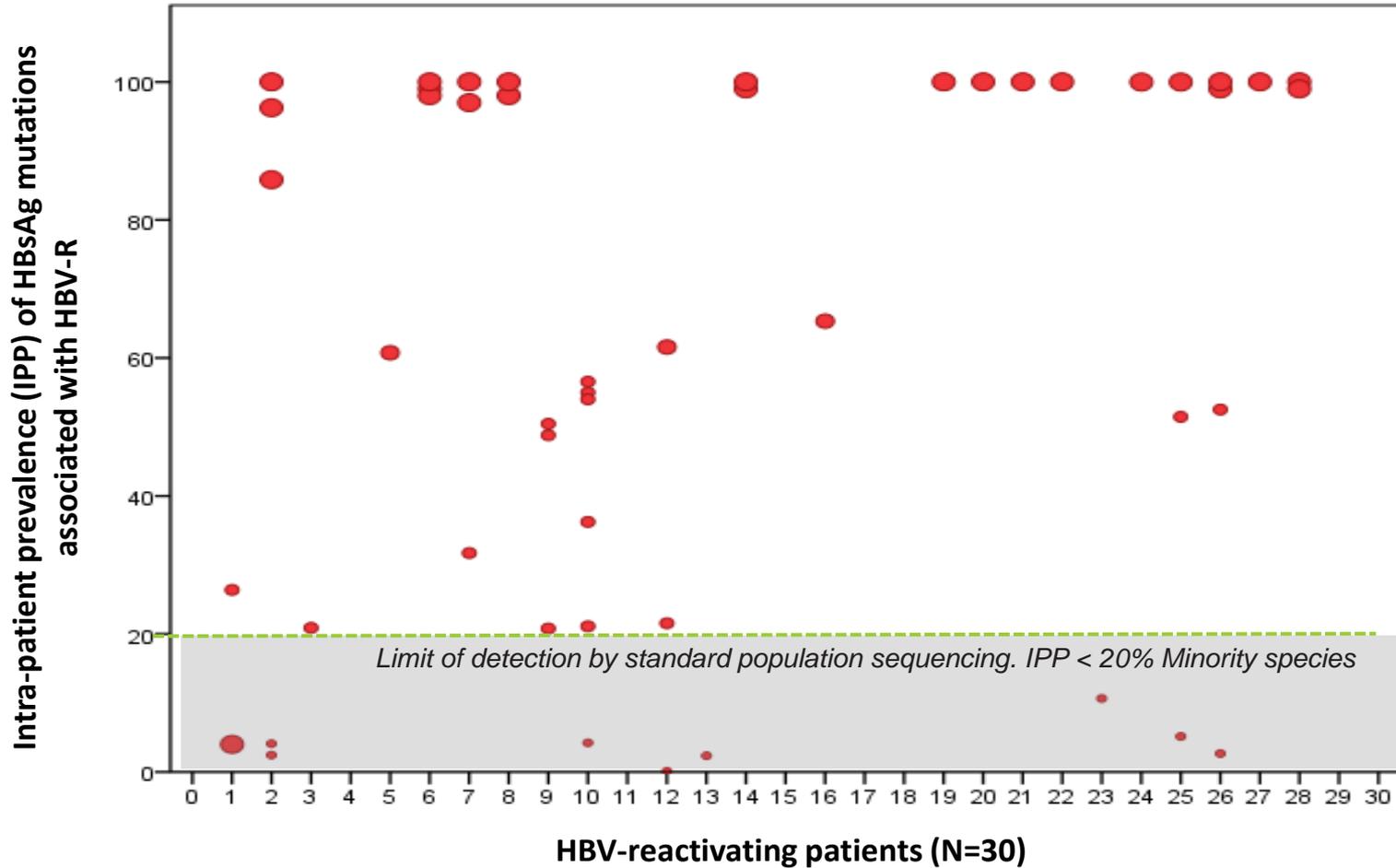


19/21 HBsAg-mutations correlated with HBV-reactivation are localized in immune active HBsAg regions:

- 15/21 reside in the **major hydrophilic loop** and are known to **hamper HBsAg recognition** by antibodies
- 4/21 are localized in **Class-I restricted T-cell epitopes**, playing a potential role in **HBV-escape** from T-mediated response.

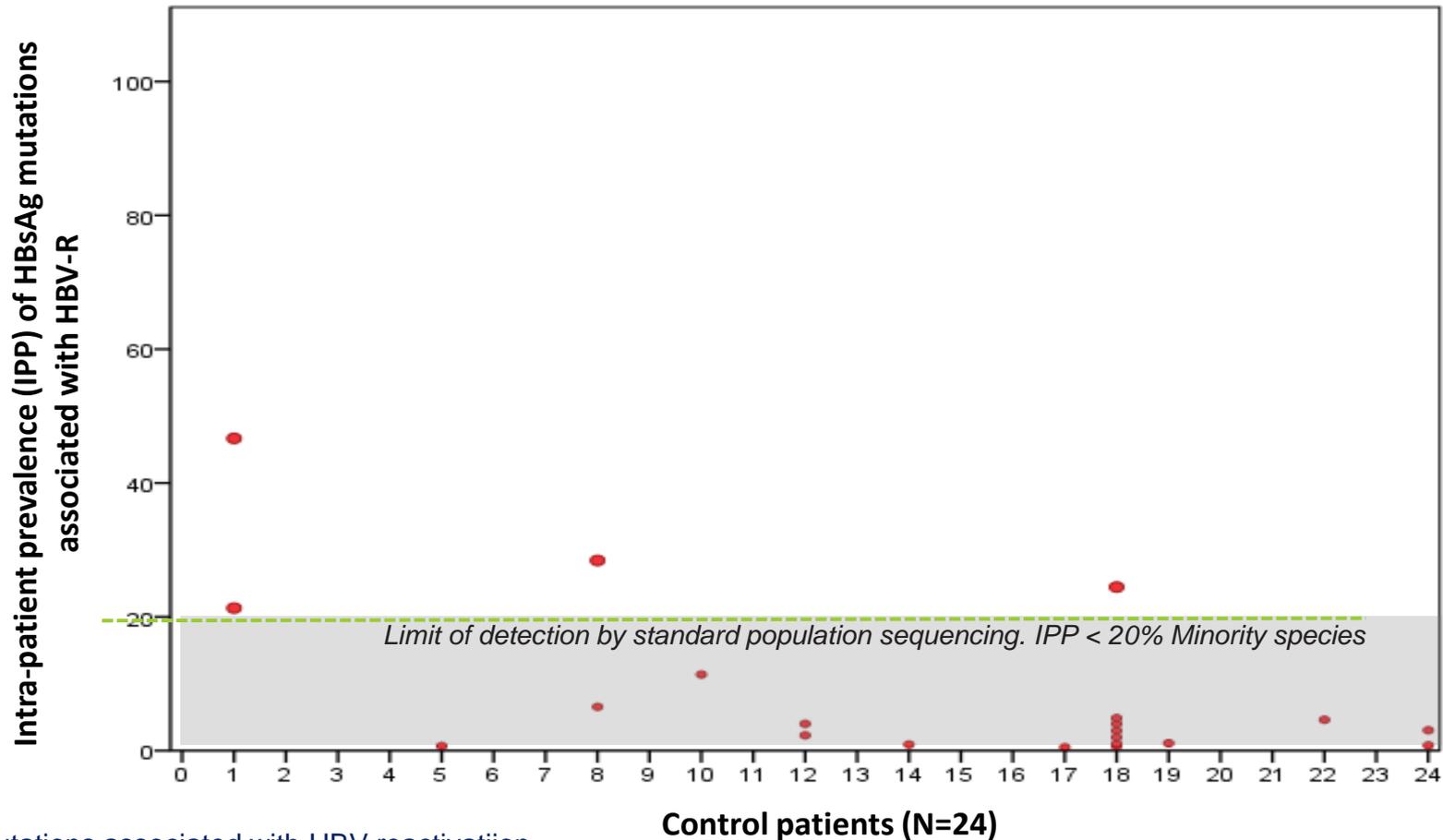


By UDPS, among HBV-reactivated patients, at least one HBsAg mutation associated with HBV-reactivation occurs always with an **intra-patient prevalence >50%, indicating their **fixation as predominant species** within HBV viral population**



● HBsAg mutations associated with HBV reactivation

In controls, HBsAg mutations associated with HBV reactivation are rare and, if detected, they are **mainly present as minor species**, never exceeding an intra-patient prevalence of 50% [ranging from 0.5 to 46.7% (P=0.008)]



● HBsAg mutations associated with HBV reactivation

Conclusions

- HBV-reactivation occurs in **a wide variety of immunosuppressive clinical settings**, and also **after completing immunosuppressive-therapy**.
- It is driven by **viral species with a peculiar variability** specifically clustered in immune active regions of HBsAg (but not in other regions of HBV genome)
- **Complex viral quasispecies**, characterizing HBV reactivation, carry HBsAg-mutations with **enhanced capability to evade B- and T- mediated immune response**

This underlines the importance of a careful patient-monitoring in all settings at reactivation risk and of establishing a prompt and potent antiviral therapy in order to prevent HBV-related clinical complications.

Thanks to.....

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