

The HBV genetic barrier and the overlapping structure of HBV genome synergistically modulate drug resistance emergence and immune escape potential of HBV genotypes

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Background

- HBV is characterized by a **high degree of genetic variability**
- This high genetic variability has allowed HBV:
 - to **spread** in different HBV genotypes
 - to **react** to the **drug-** and **immune-**pressure by acquiring mutations in the **RT** (the only target of the currently available **anti-HBV drugs**) and the **HBsAg** (target of the **neutralizing antibodies**)

Objective

- To define the impact **in different HBV genotypes** of **genetic barrier** defined as the number and type of nucleotide substitutions required to overcome drug- and immune- pressure.
 - This is the first study in the field of HBV addressing this point.

Methods

- This study includes **3,482** HBV-RT/HBsAg sequences from 555 drug-naive and 2,927 antiviral-treated patients, infected with HBV-genotype **A** (N=542), **B** (N=607), **C** (N=1332), **D** (N=689), **E** (N=198), **F** (N=74), **G** (N=42).
- The genetic-barrier was defined as the sum of nucleotide-**transitions** (**score=1**) and/or nucleotide-**transversions** (**score=2.5**) required for the evolution from the wild-type codon to a specific drug-resistance or immune-escape mutations (*Van der Vijer et al.,2006*).
- We analyzed:
 - **16 RT codons** associated with **drug-resistance**:
80, 84, 169, 173, 180, 181, 184, 191, 194, 202, 204, 207, 215, 233, 236, 250
 - **16 HBsAg codons** associated with **immune/diagnostic-escape**:
114, 118, 120, 123, 126, 127, 128, 129, 130, 131, 133, 141, 144, 145, 158, 161

Despite a natural variability, the genetic-barrier for drug-resistance development was identical among all HBV-genotypes

WT RT amino acid	WT RT codon	HBV Genotype							Resistance mutation	Preferred Resistance Codon	Lowest score
		A N = 67	B N= 124	C N = 189	D N = 140	E N = 13	F N = 14	G N = 8			
Mutations associated with resistance to Lamivudine and Telbivudine											
M204	ATG	60 (89.5)	120 (96.8)	175 (92.6)	140 (100)	13 (100)	13 (92.9)	8 (100)	M204I	ATA	1
M204	ATG	60 (89.5)	120 (96.8)	175 (92.6)	140 (100)	13 (100)	13 (92.9)	8 (100)	M204V	GTG	1
Mutations associated with resistance to Entecavir											
I169	ATT	1 (1.5)	11 (8.9)	184 (97.4)	129 (92.1)	13 (100)	1 (7.1)	0 (0.0)	I169T	ACT	1
	ATA	66 (98.5)	111 (89.5)	4 (2.1)	7 (5.0)	0 (0.0)	13 (92.9)	8 (100)	I169T	ACA	1
	ATC	0 (0.0)	2 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	I169T	ACC	1
T184	ACT	67 (100)	124 (100)	189 (100)	140 (100)	13 (100)	14 (100)	8 (100)	T184G	GGT	2.5
S202	AGT	5 (7.5)	124 (100)	187 (98.9)	132 (94.3)	13 (100)	13 (92.9)	1 (12.5)	S202G	GGT	1
	AGC	62 (92.5)	0 (0.0)	2 (1.1)	6 (42.9)	0 (0.0)	1 (7.1)	7 (87.5)	S202G	GGC	1
M250	ATG	67 (100)	124 (100)	189 (100)	140 (100)	13 (100)	14 (100)	8 (100)	M250V	GTG	1
Mutations associated with cross-resistance to Lamivudine and Adefovir											
A181	GCT	67 (100)	124 (100)	187 (98.9)	136 (97.1)	13 (100)	14 (100)	8 (100)	A181T	ACT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	A181T	ACC	1
A181	GCT	67 (100)	124 (100)	187 (98.9)	136 (97.1)	13 (100)	14 (100)	8 (100)	A181V	GTT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	A181V	GTC	1
A181	GCT	67 (100)	124 (100)	187 (98.9)	136 (97.1)	13 (100)	14 (100)	8 (100)	A181S	ACT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	A181S	ACC	1
Mutations associated with resistance to Adefovir											
N236	AAC	67 (100)	123 (99.2)	179 (94.7)	129 (92.1)	2 (15.4)	2 (14.3)	8 (100)	N236T	ACC	2.5
	AAT	1 (1.5)	1 (0.8)	10 (5.3)	11 (7.9)	11 (84.6)	12 (85.7)	0 (0.0)	N236T	ACT	2.5
Mutations associated with resistance to Tenofovir											
A194	GCT	67 (100)	123 (99.2)	189 (100)	135 (96.4)	13 (100)	14 (100)	8 (100)	A194T	ACT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	A194T	ACC	1

Despite a natural variability, the genetic-barrier for drug-resistance development was identical among all HBV-genotypes

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		A N = 67	B N= 124	C N = 189	D N = 140	E N = 13	F N = 14	G N = 8			
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M204	ATG	60 (89.5)	120 (96.8)	175 (92.6)	140 (100)	13 (100)	13 (92.9)	8 (100)	M204I	ATA	1
M204	ATG	60 (89.5)	120 (96.8)	175 (92.6)	140 (100)	13 (100)	13 (92.9)	8 (100)	M204V	GTG	1
Mutations associated with resistance to Entecavir											
I169	ATT	1 (1.5)	11 (8.9)	184 (97.4)	129 (92.1)	13 (100)	1 (7.1)	0 (0.0)	I169T	ACT	1
	ATA	66 (98.5)	111 (89.5)	4 (2.1)	7 (5.0)	0 (0.0)	13 (92.9)	8 (100)	I169T	ACA	1
	ATC	0 (0.0)	2 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	I169T	ACC	1
T184	ACT	67 (100)	124 (100)	189 (100)	140 (100)	13 (100)	14 (100)	8 (100)	T184G	GGT	2.5
S202	AGT	5 (7.5)	124 (100)	187 (98.9)	132 (94.3)	13 (100)	13 (92.9)	1 (12.5)	S202G	GGT	1
	AGC	62 (92.5)	0 (0.0)	2 (1.1)	6 (42.9)	0 (0.0)	1 (7.1)	7 (87.5)	S202G	GGC	1
M250	ATG	67 (100)	124 (100)	189 (100)	140 (100)	13 (100)	14 (100)	8 (100)	M250V	GTG	1
Mutations associated with cross-resistance to Lamivudine and Adefovir											
A181	GCT	67 (100)	124 (100)	187 (98.9)	136 (97.1)	13 (100)	14 (100)	8 (100)	A181T	ACT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	A181T	ACC	1
A181	GCT	67 (100)	124 (100)	187 (98.9)	136 (97.1)	13 (100)	14 (100)	8 (100)	A181V	GTT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	A181V	GTC	1
A181	GCT	67 (100)	124 (100)	187 (98.9)	136 (97.1)	13 (100)	14 (100)	8 (100)	A181S	ACT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	A181S	ACC	1
Mutations associated with resistance to Adefovir											
N236	AAC	67 (100)	123 (99.2)	179 (94.7)	129 (92.1)	2 (15.4)	2 (14.3)	8 (100)	N236T	ACC	2.5
	AAT	1 (1.5)	1 (0.8)	10 (5.3)	11 (7.9)	11 (84.6)	12 (85.7)	0 (0.0)	N236T	ACT	2.5
Mutations associated with resistance to Tenofovir											
A194	GCT	67 (100)	123 (99.2)	189 (100)	135 (96.4)	13 (100)	14 (100)	8 (100)	A194T	ACT	1
	GCC	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	A194T	ACC	1

Despite a natural variability, the genetic-barrier for drug-resistance mutations is the same among the different HBV-genotypes

WT RT amino acid	WT RT Codon	HBV Genotype							Resistance mutation	Minimal Score
		A N = 67	B N = 124	C N = 189	D N = 140	E N = 13	F N = 14	G N = 8		
L80	TTA	64 (95.5)	118 (95.2)	15 (7.9)	128 (91.4)	2 (15.4)	0 (0.0)	2 (25.0)	L80V	2.5
	CTA	2 (3.0)	2 (1.6)	172 (91.0)	12 (8.6)	11 (84.6)	14 (100)	6 (75.0)	L80V	2.5
V173	GTG	67 (100)	124 (100)	188 (99.5)	136 (97.1)	13 (100)	14 (100)	8 (100)	V173L	2.5
L180	CTG	3 (4.5)	3 (2.4)	169 (89.4)	125 (89.3)	12 (92.3)	12 (85.7)	1 (12.5)	L180M	2.5
	TTG	59 (88.1)	119 (96.0)	7 (3.7)	13 (9.3)	1 (7.7)	1 (7.1)	7 (87.5)	L180M	2.5
	CTC	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	L180M	5
	CTA	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	L180M	3.5
Q215	CAG	51 (76.1)	0 (0.0)	0 (0.0)	83 (59.3)	2 (15.4)	13 (92.9)	1 (12.5)	Q215S	3.5
	CAA	15 (22.4)	123 (99.2)	188 (99.5)	33 (23.6)	11 (84.6)	1 (7.1)	7 (87.5)	Q215S	3.5



Conversely....

**HBV genetic barrier varies according
to the type of drug-resistance mutations**

Indeed....

The genetic barrier for **primary mutations** is significantly **lower** than that observed for **secondary mutations**

	Genetic Barrier Score Median (min-max)	P Value
Primary mutations	1.0 (1.0-2.5)	0.011
Secondary mutations	2.5 (1.0-5.0)	

Primary mutations considered are: A181T/V, A194T, M204V/I, N236T, while secondary mutations are: L80I/V, I169T, V173L, L180M, T184G/S, Q215S, S202I, M250V (*Zoulim and Locarnini , Gastroenterology 2009*)

P value was determined by Mann-Whitney test.



In case of **immune-escape**, genetic-barrier for development of specific **HBsAg-mutations** varies **among HBV-genotypes**.

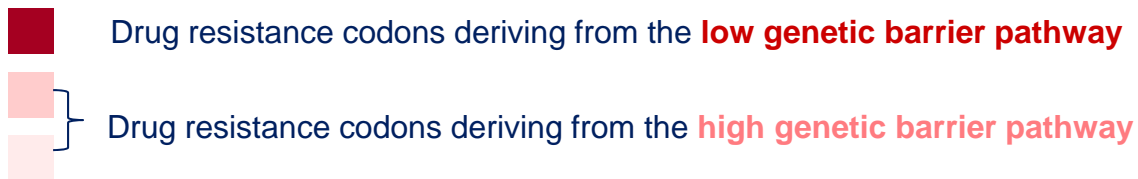
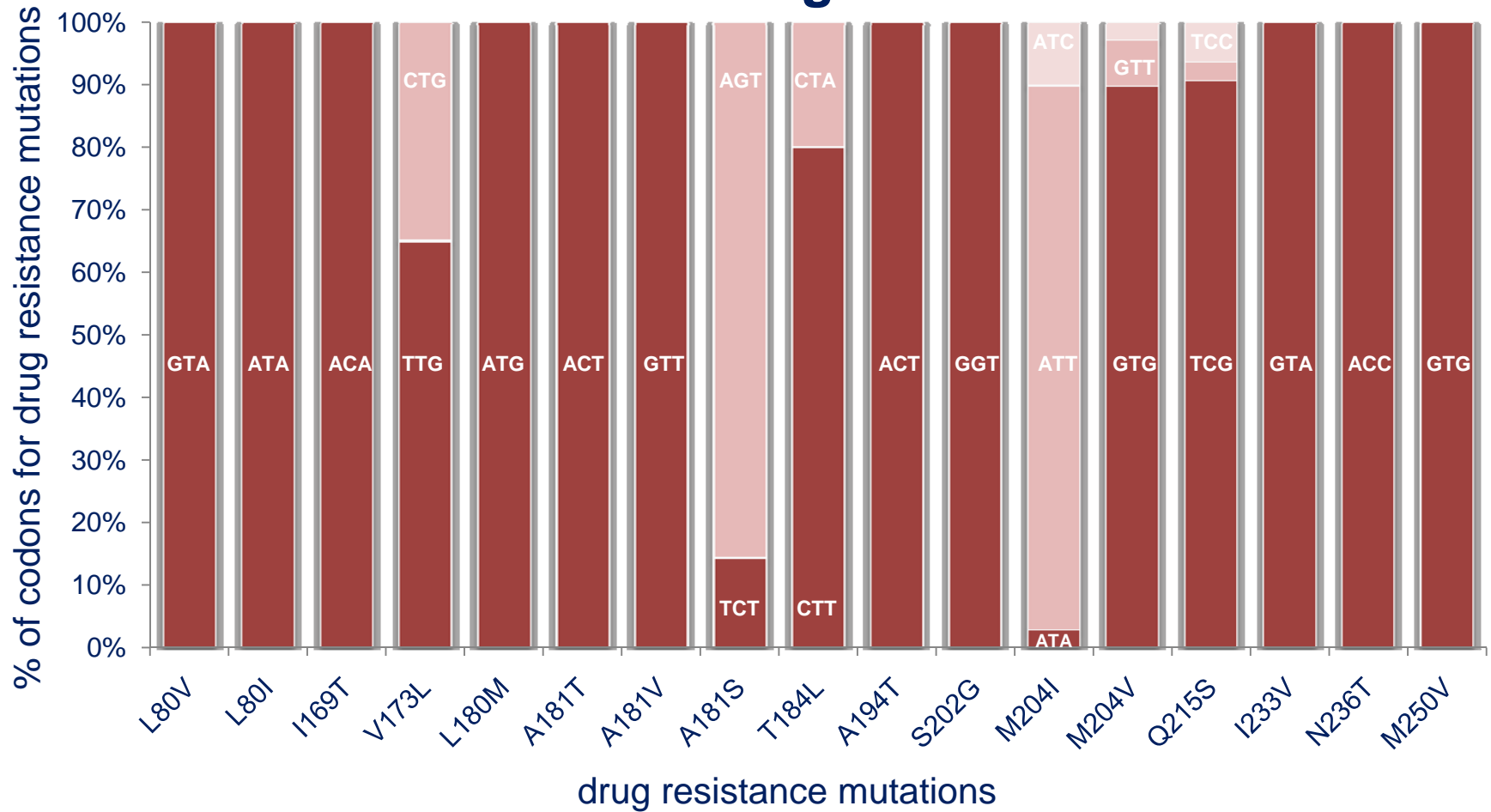
HBV genotypes **A** and **G** are more prone to develop the immune/diagnostic-escape mutations **sT114r** and **sG130N**

WT HBsAg position	HBV Genotype							Immune-escape mutation	Minimal Score
	A N = 67	B N = 124	C N = 189	D N = 140	E N = 13	F N = 14	G N = 8		
S114 TCA	1 (1.5)	121(97.6)	183(96.8)	128(91.4)	13 (100)	2 (14.3)	0 (0.0)	S114R CGA	3.5
TCG	1 (1.5)	2 (1.6)	0 (0.0)	6 (42.9)	0 (0.0)	0 (0.0)	7 (87.5)	S114R CGG	3.5
T114 ACA	65 (97.0)	0 (0.0)	1 (0.5)	3 (2.1)	0 (0.0)	1 (7.1)	1 (12.5)	T114R AGA	2.5
ACG	1 (1.5)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	11(78.6)	0 (0.0)	T114R AGA	3.5
G130 GGA	0 (0.0)	122 (98.4)	186(98.4)	128 (91.4)	13 (100)	13(92.9)	0 (0.0)	G130N AAT/C	4.5
G130 GGC	67 (100)	2 (1.6)	2 (1.1)	6 (4.3)	0 (0.0)	0 (0.0)	8 (100)	G130N AAC	2

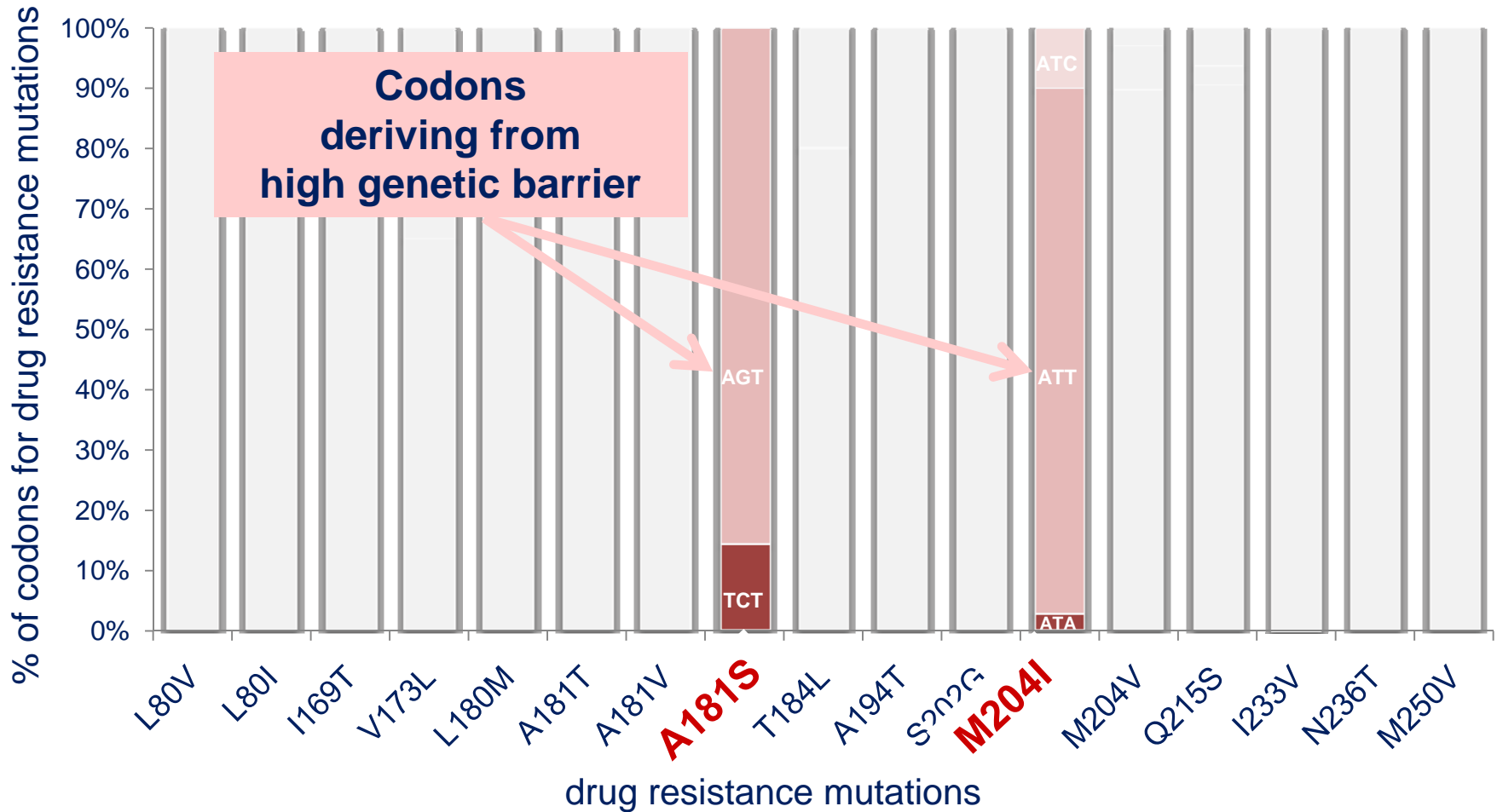
The Asparagine (N) at the HBsAg position 131, associated in vitro with vaccine escape, is the wild-type amino acid in both genotypes **A** and **G**




WT HBsAg position	HBV Genotype							Immune-escape mutation	Minimal Score	
	A N = 67	B N = 124	C N = 189	D N = 140	E N = 13	F N = 14	G N = 8			
T131	ACC	4 (6.0)	122 (98.4)	188 (99.4)	132 (94.3)	13 (100)	14 (100)	0 (0.0)	T131N AAC	2.5
	ACA	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	T131N AAT/C	5
N131	AAC	66 (98.5)	2 (1.6)	1 (0.5)	6 (4.3)	0 (0.0)	0 (0.0)	8 (100)	-	0

Drug-resistance development follows the pathway with the lowest genetic-barrier

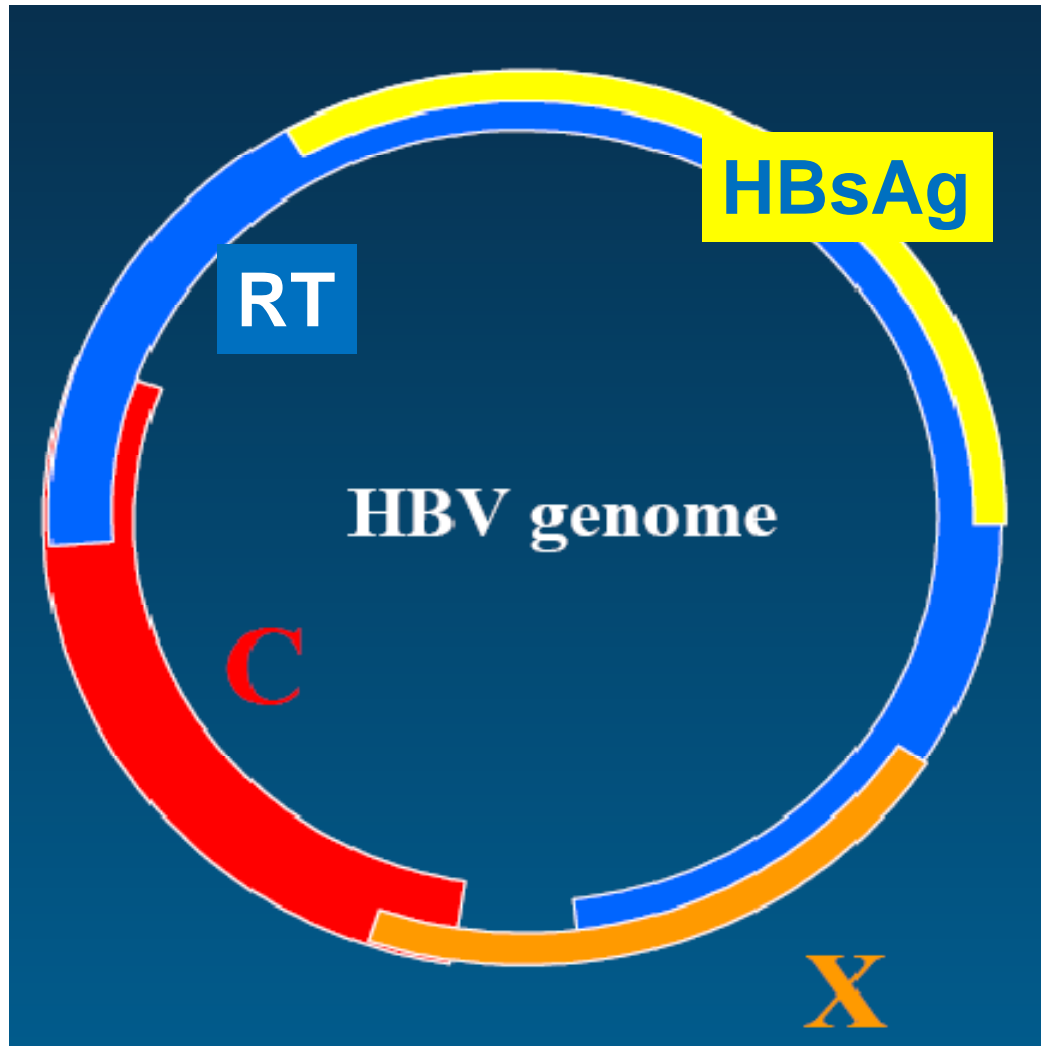


There are only two exceptions: **rtM204I** and **rtA181S**

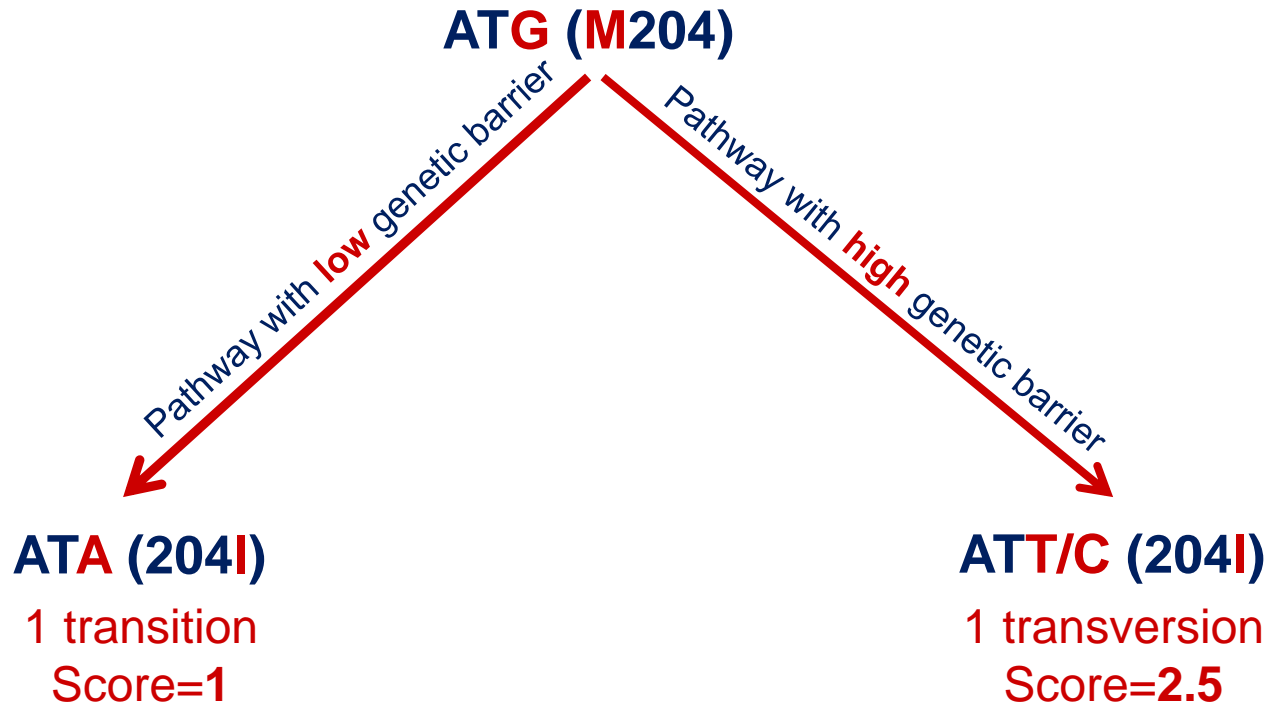


-  Drug resistance codons deriving from the low genetic barrier pathway
-   Drug resistance codons deriving from the high genetic barrier pathway

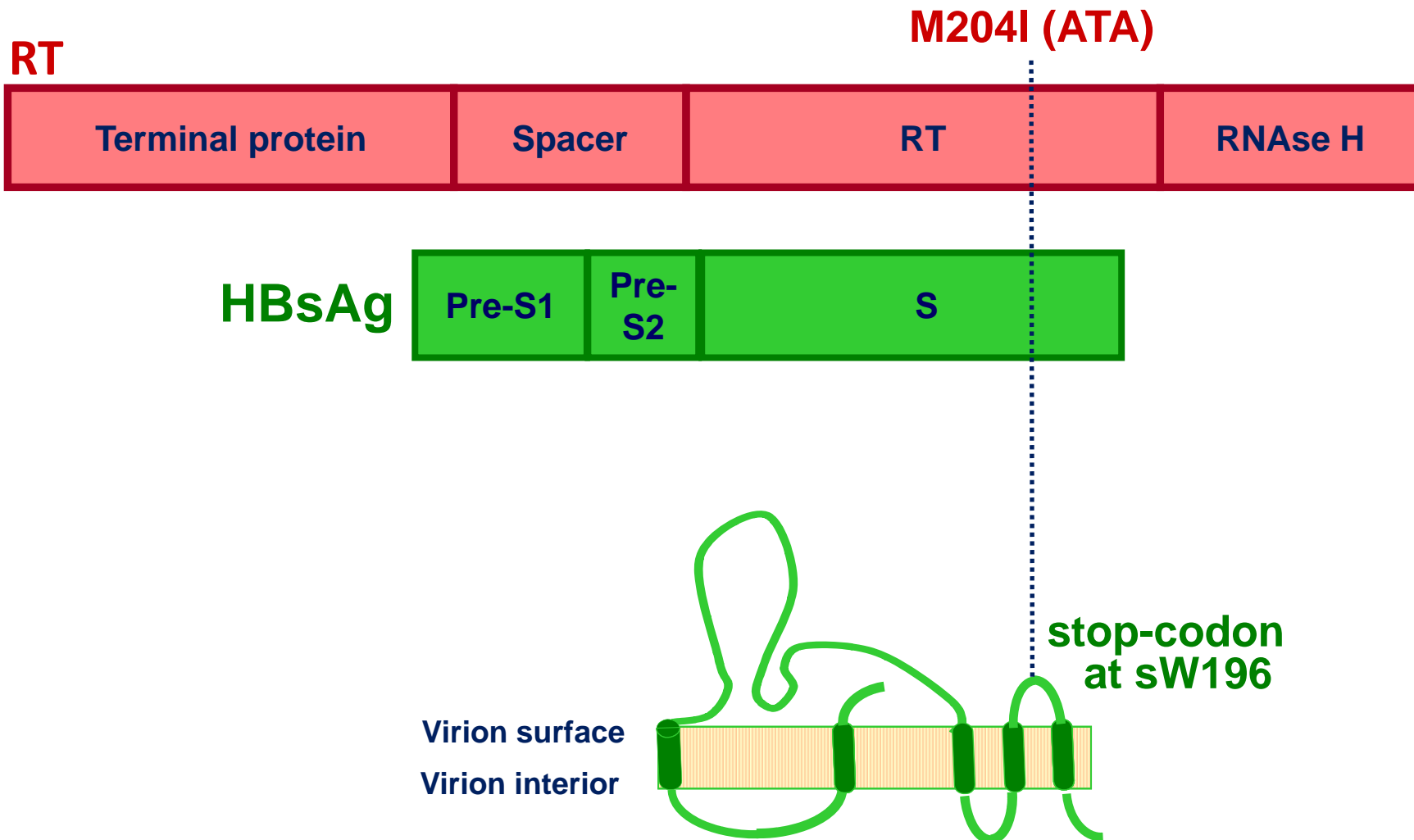
The different codon usage can be explained by HBV genome organization and in particular by the overlapping between the RT and HBsAg genes



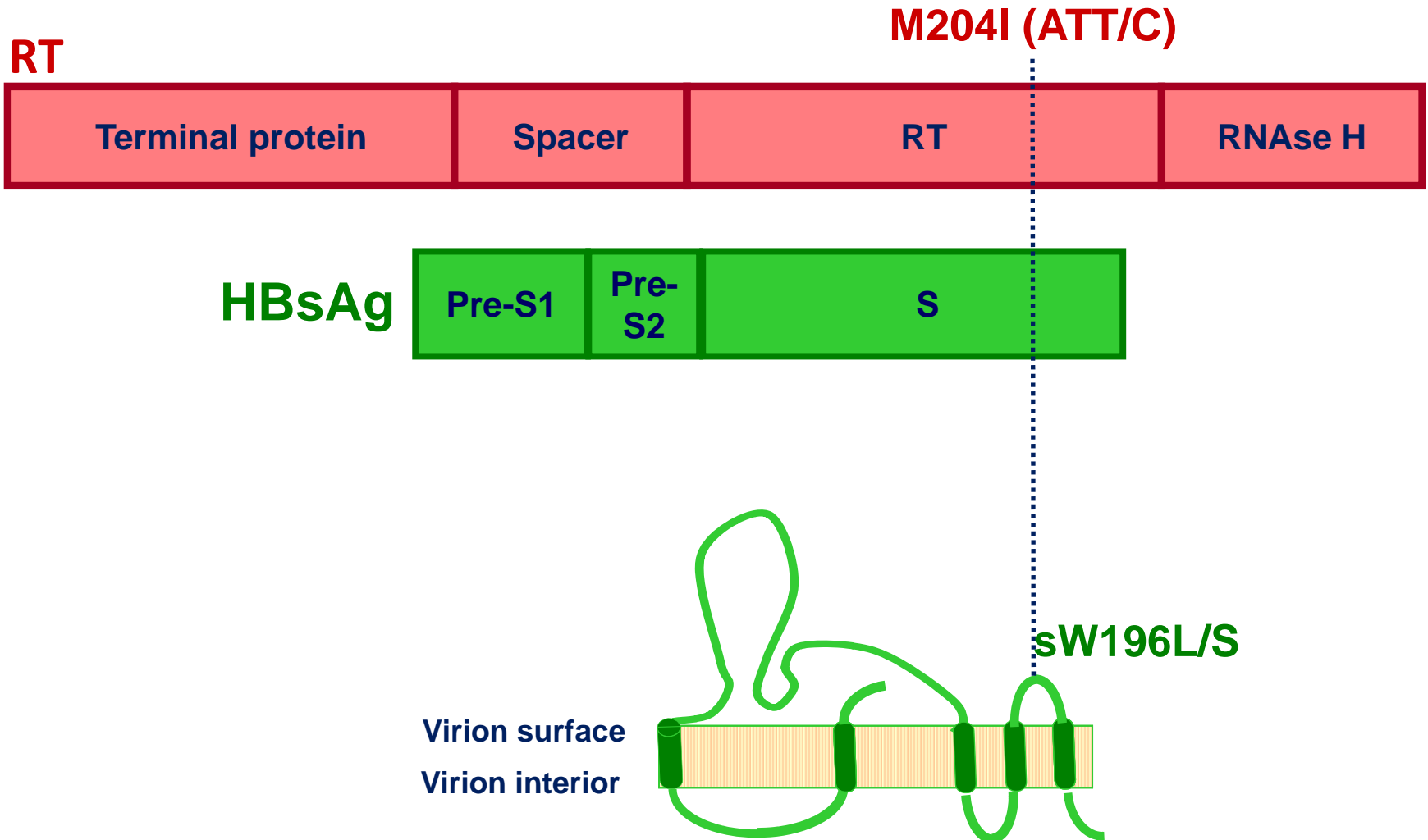
Genetic Pathways for the evolution to M204I



Due to the overlapping between the RT and the HBsAg encoding sequences, the transition (associated with low genetic-barrier) introducing a stop-codon in the HBsAg-position sW196



Transversions do not introduce any stop codon in the HBsAg and thus they are preferred for 204I, and commonly detected at virological failure





Overall results support the paradigm of “***Constrained HBV evolution***”:

- the overlapping between genes can constrain and limit the genetic variability that HBV can tolerate

Conclusions

- Our study shows that the genetic-barrier and the RT/HBsAg overlapping influence HBV drug-resistance and immune-escape development.
- The different immune-escape potential of specific HBV-genotypes can have important clinical consequences in terms of disease progression, vaccine strategies, and correct HBsAg detection.

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