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Barcelona**

Abst#_10

**Analysis of genetic and viral determinants
of HBsAg levels in patients with chronic infection**

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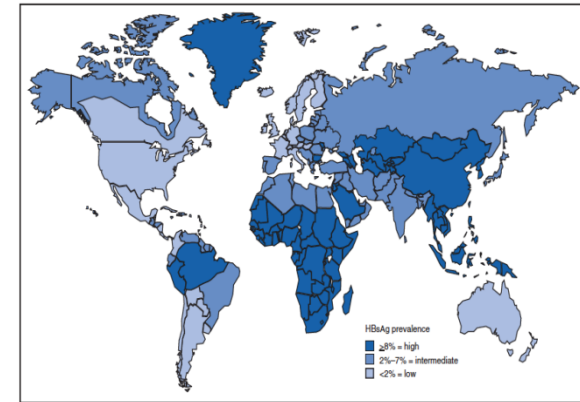


❑ Amount of HBV-DNA levels in serum :

- marker of virological response to treatment
- prognostic marker of the disease

❑ Amount of HBsAg (qHBsAg):

- predictor of response to PEG-IFN treatment
- predictor of risk of disease progression



❑ HBV genotype:

- best response to PEG-IFN therapy (genotypes A e B)

❑ Single Nucleotide Polimorphisms (SNPs) of IL28B, rs12979860 and rs8099917:

- correlates of sustained virological response (SVR) in HCV chronic hepatitis treated with PegIFN/RBV

❑ From recent literature a plausible role of the SNPs of IL28B in HBV infection:

- Clearance of HBsAg, spontaneous or induced by IFN therapy
- HBeAg seroconversion induced by IFN therapy
- Disease activity

Study aims

- ❑ To analyze the distribution of 6 SNPs of IL28B:
rs12980275, rs8105790, rs12979860, rs8099917, rs7248668, rs10853728 in patients with chronic HBV infection
- ❑ To verify their correlation with levels of HBsAg and HBV-DNA
- ❑ To identify other predictors of levels of HBsAg and HBV-DNA
- ❑ To explore the changes of levels of HBsAg over a period of 6 months

Methods

- Patients with HBsAg + > 6 months, naïve or on treatment with NAs
- HBV-DNA quantification : Abbott Real Time HBV, sensitivity 10 IU/mL
- HBsAg quantification : Abbott Architect HBsAg, sensitivity 0.05 IU/mL
- Genotyping and analysis of mutations involved in drug resistance:
 - Sequencing of an HBV fragment of about 1000bp in the P region (polymerase)
- Sequencing of human IL28B region with primer pairs specific for each SNP

SNPs	Localization	Sequence [SNPs]
rs12980275	19:39731783	TGAGAGAAGTCAAATTCCTAGAAAC[A/G]GACGTGTCTAAATATTTGCCGGGGT
rs8105790	19:39732501	TTCCTGACATCACTCCAATGTCCTG[C/T]TTCTGTGGTTACATCTTCCGCTAAT
rs12979860	19:39738787	TGAACCAGGGAGCTCCCCGAAGGCG[C/T]GAACCAGGGTTGAATTGCACTCCGC
rs8099917	19:39743165	TTTTGTTTTCTTTCTGTGAGCAAT[G/T]TCACCCAAATTGGAACCATGCTGTA
rs7248668	19:39743821	ATGGTCTCAGTCTGTAGCCCAAGCT[A/G]GAGCATAGTAGTGGCACAATCGCCA
rs10853728	19:39745146	CGTAAGCAGCCTGGGAGATGTGGGC[C/G]TAAGCTTTGGTGAGGATGAGAGTCT

Statistical analysis:

- Anova Test for correlations between SNPs and qHBsAg and HBV-DNA
- Linear regression for correlates of qHBsAg and HBV DNA
- T Student test to analyze qHBsAg changes over 6 months

Descriptive characteristics of all HBV-infected patients: n° 70

Age (years)*	45.4 (33.2-58.0)
Gender	M 46 (65.7%)
Ethnicity	Caucasian 56 (80%)
BMI (kg/m ²)*	23.8 (21.7-28.4)
Time from diagnosis of HBV infection (years)*	5.3 (2.2-12.5)
Coinfection HDV	2 (2.9%)
Coinfection HCV	1 (1.4%)
Coinfection HIV	3 (4.3%)
Genotype	D 39 (55.7%), A 9 (12.9%), E 3 (4.3%) B 1 (1.4%), C 2 (2.9%), unknown 16 (22.9%)
HBeAg +	9 (12.9%)
NAs therapy	31 (44.3%)
Entecavir	23 (74.2%)
Tenofovir	7 (22.6%)
Lamivudine	1 (3.2%)
HBV-DNA <10 IU/mL	23 (32.9%)
qHBsAg, log IU/ml*	3.2 (2.2-3.9)
HBV-DNA, log IU/ml*	2.2 (0-3.3)
ALT, UI/l*	26 (16.7-34.2)
Inactive carrier status, N. (%)	26 (37%)
Cirrhosis	8 (11.4%)

Values expressed in n(%), except* median (IQR)

SNPs in linkage disequilibrium

SNPs

rs12979860	CC	CT	TT
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n° (%)	26(37.1)	30(42.9)	14(20)
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rs8105790	TT	CT	CC
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n° (%)	41(58.6)	27(38.6)	2(2.9)
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rs12980275	GG	GA	AA
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n° (%)	13(18.6)	32(45.6)	25 (35.7)
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rs8099917	TT	GT	GG
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n° (%)	43(61.4)	25(35.7)	2(2.9)
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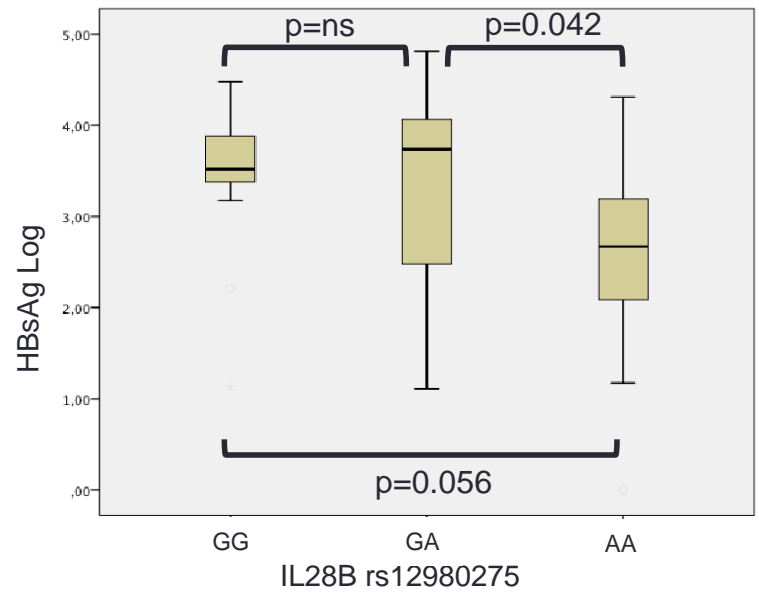
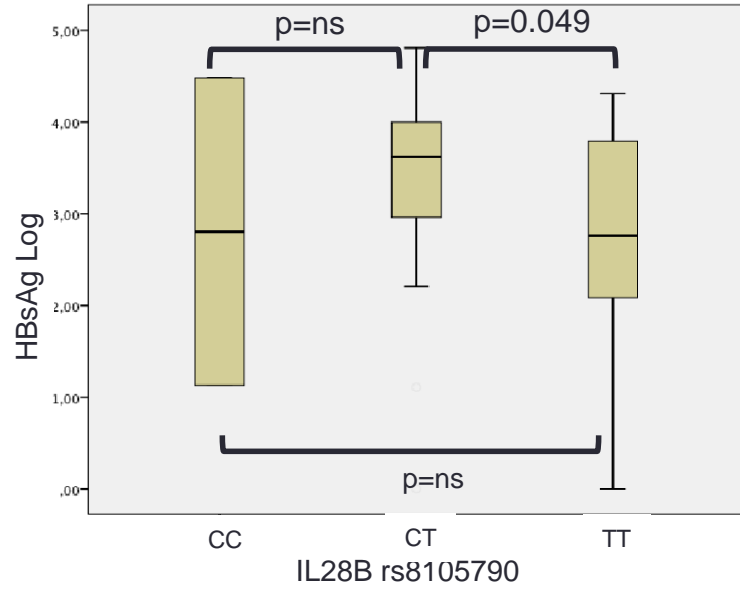
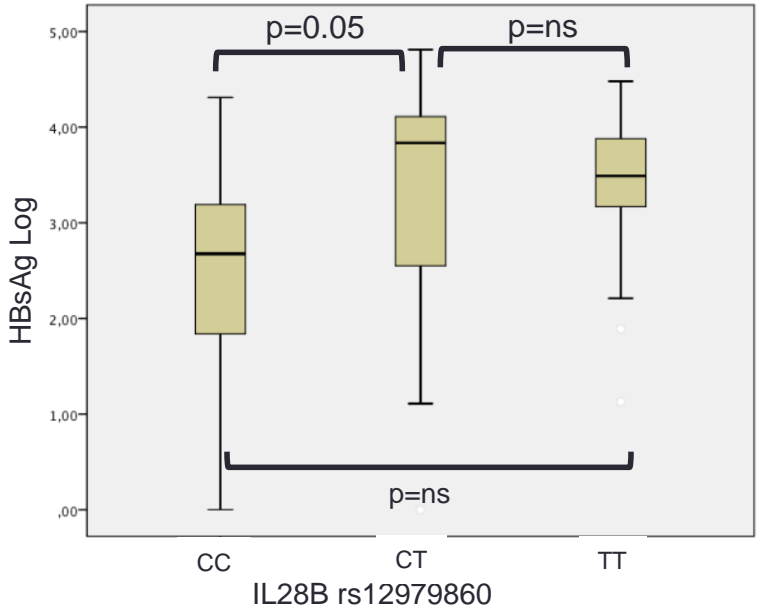
rs7248668	GG	AG	AA
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n° (%)	44(62.9)	24 (34.2)	2 (2.9)
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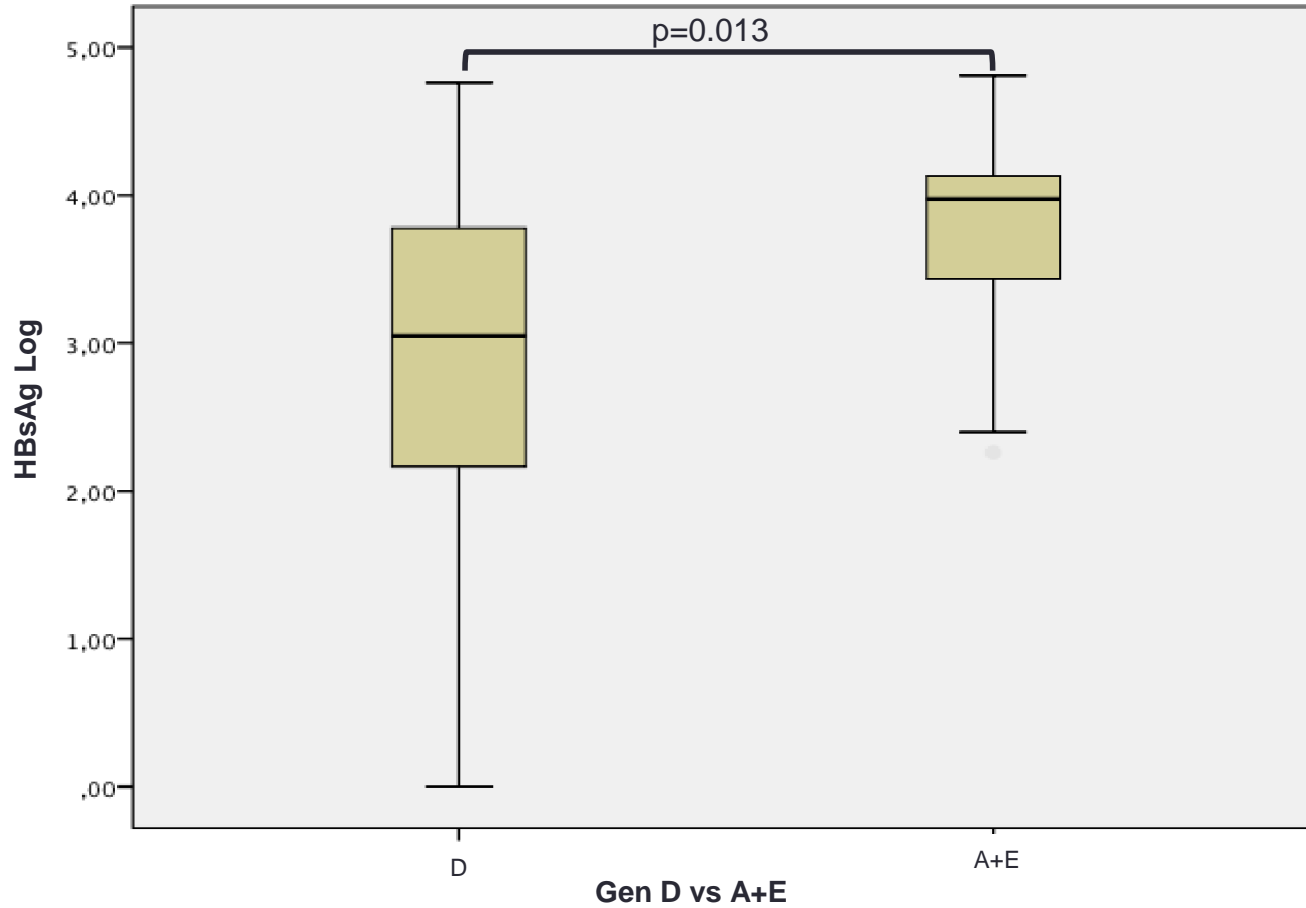
rs10853728	CC	CG	GG
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n° (%)	7(10)	29 (41.4)	34 (48.6)
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Correlation of the SNPs with qHBsAg



Role of viral genotype on qHBsAg



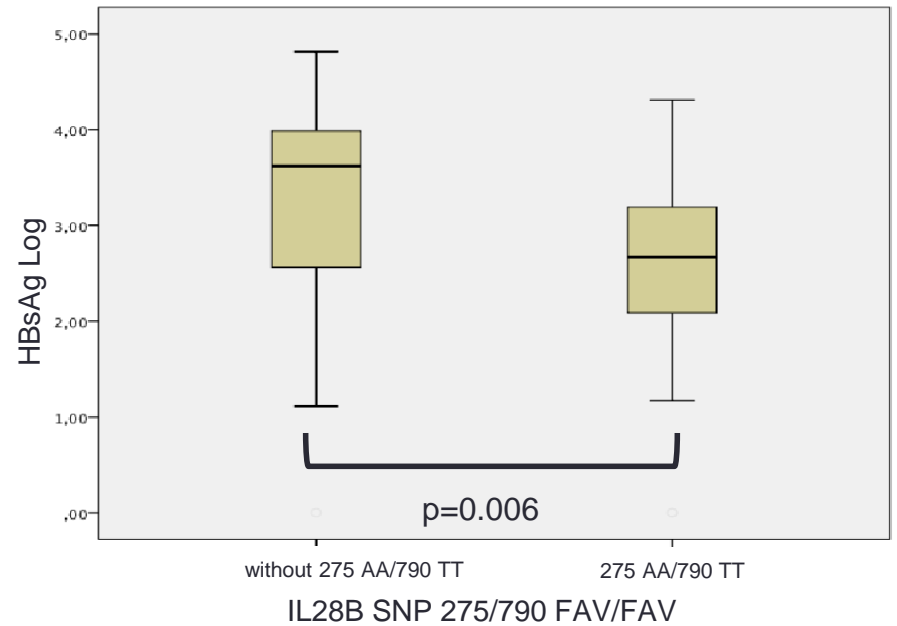
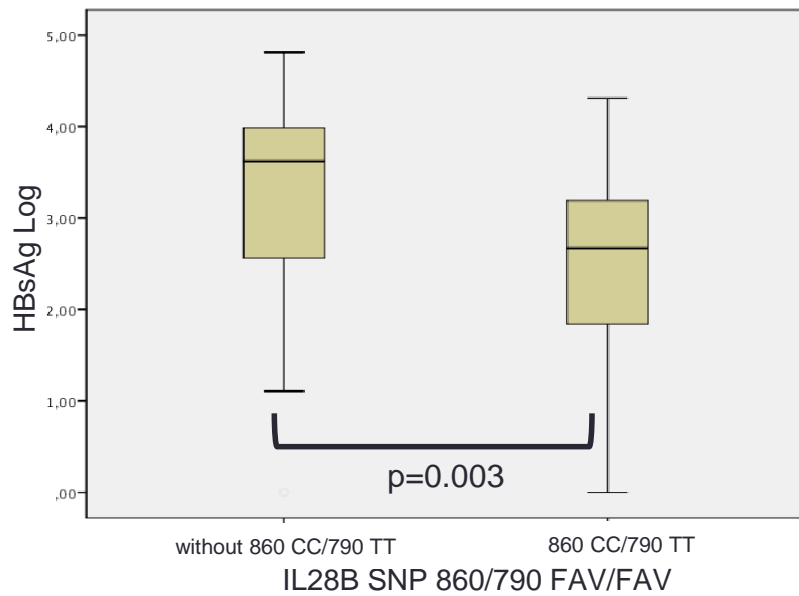
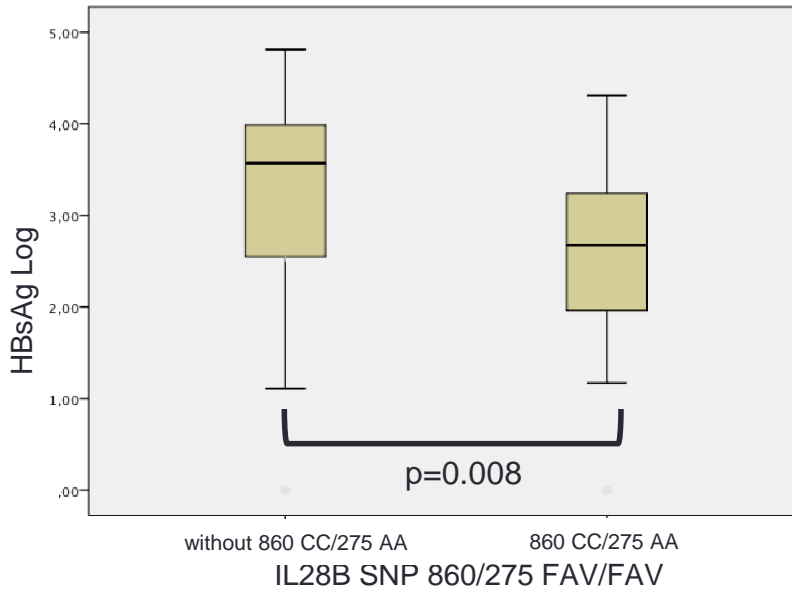
Multivariate analysis

	P value	B (95%CI) qHBsAg
II28B 860 CC vs CT	0.08	-0.08 (-0.17;0.01)
Gen A+E vs D	0.33	+0.44 (-0.47;1.35)

	P value	B (95%CI) qHBsAg
II28B 275 AA vs GA	0.06	-0.68 (-1.41;0.05)
Gen A+E vs D	0.34	+0.43 (-0.48;1.34)

	P value	B (95%CI) qHBsAg
II28B 790 TT vs CT	0.01	-0.71 (-0.14;-1.289)
Gen D vs A+E	0.01	-0.86 (-0.20;-1.52)

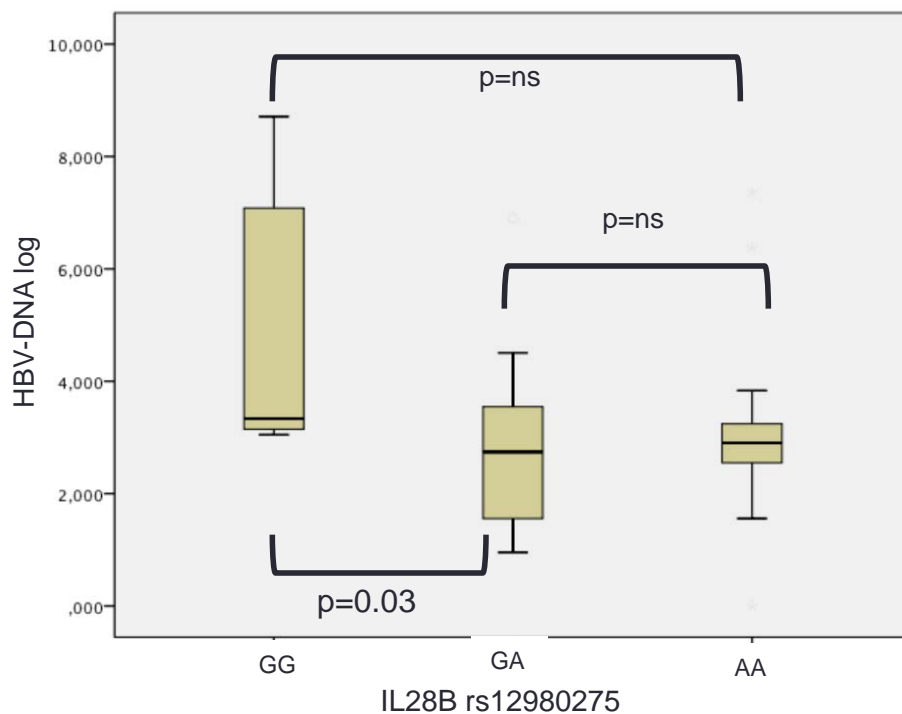
Role of favourable haplogroups on qHBsAg (n=70)



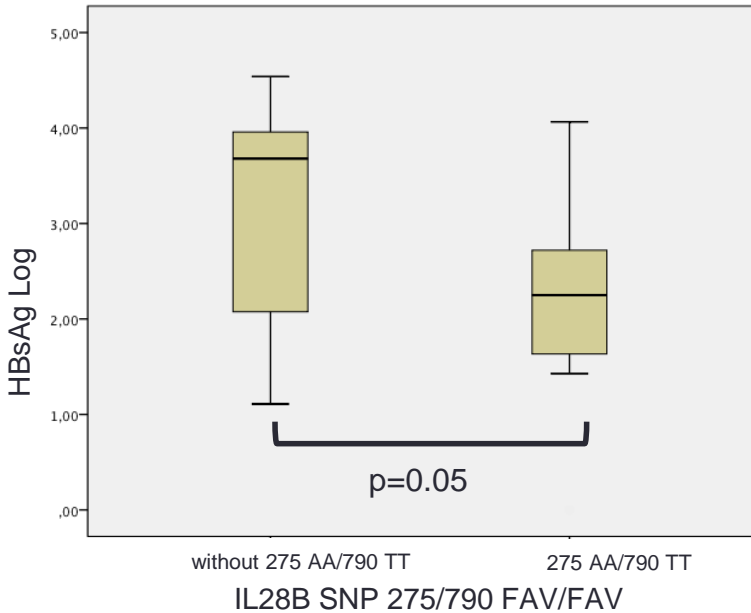
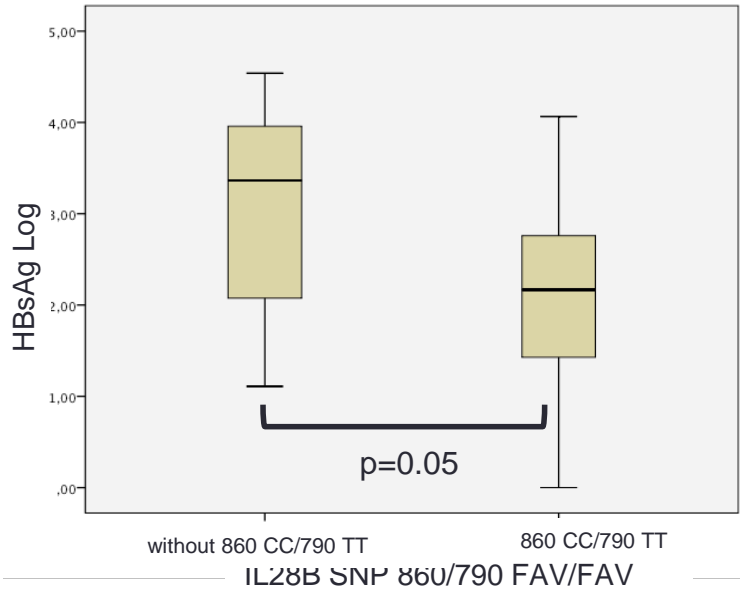
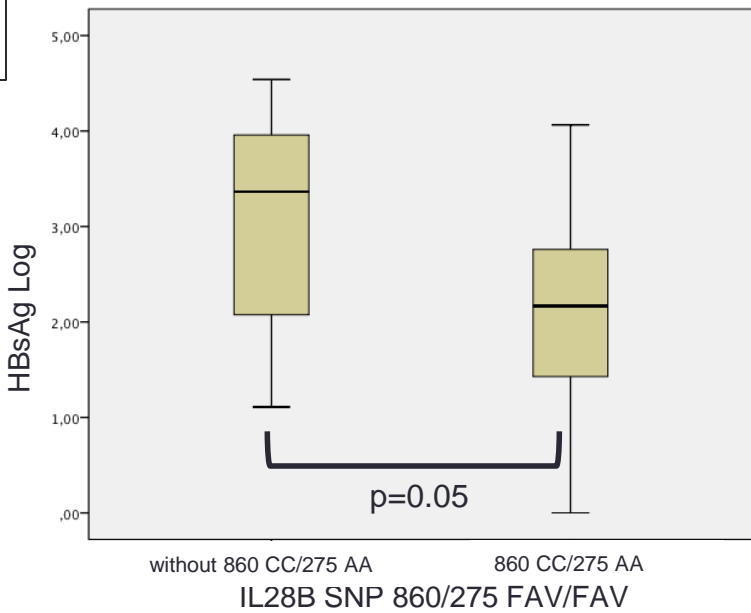
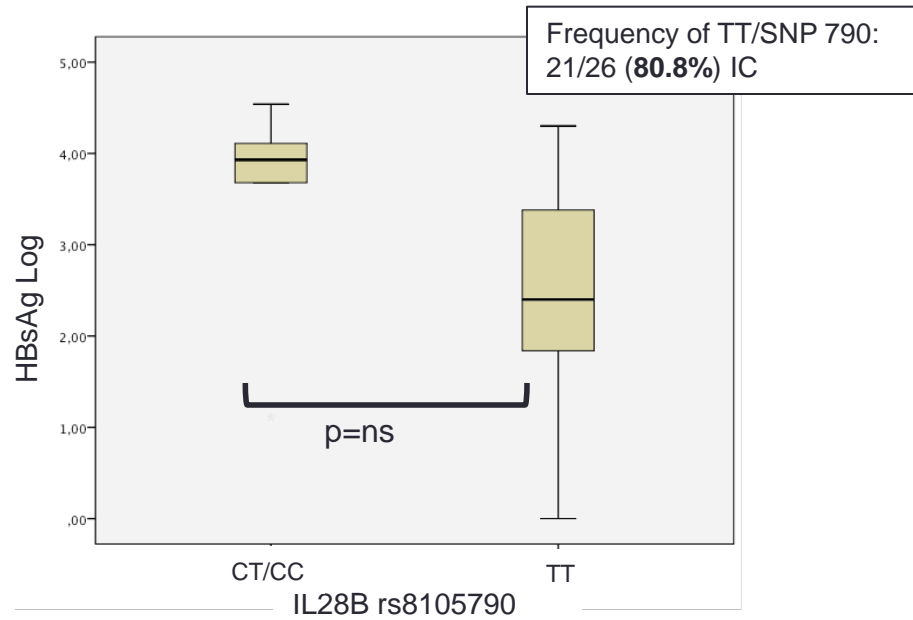
Correlation of the SNPs with HBV-DNA

- No significant correlations of the SNPs with HBV DNA were found in all patients (n=70)

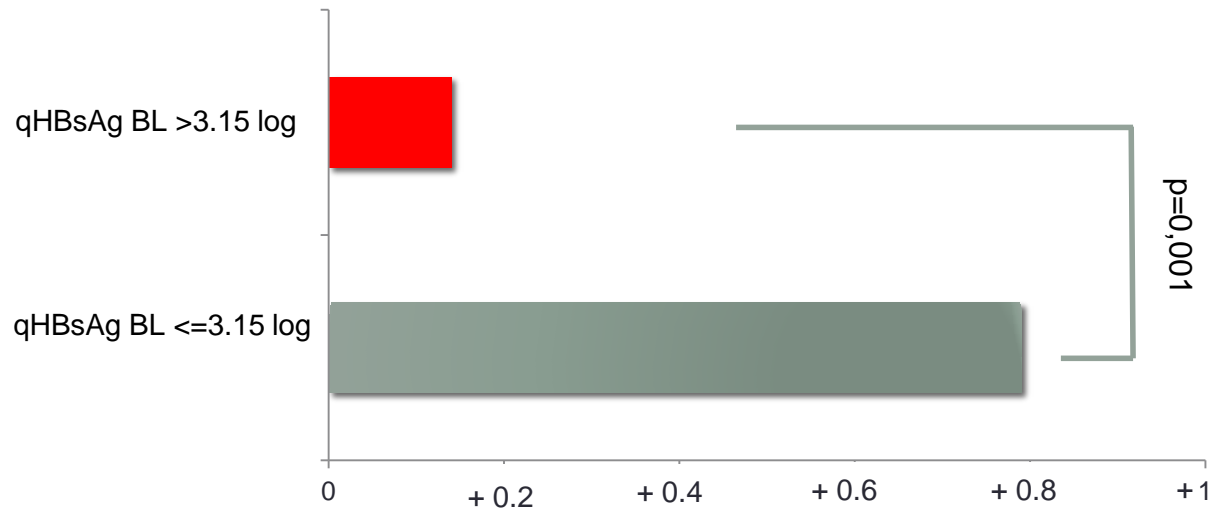
Correlation of the SNPs with HBV-DNA in untreated patients (n= 39)



Univariate and multivariate analysis (HBsAg): Inactive Carriers (n= 26)



Change of qHBsAg over 6 months



Change from baseline of qHBsAg (log IU/mL)

Conclusions

- ✓ Variability of qHBsAg is associated with SNPs in IL28B
- ✓ These variants influence levels of HBsAg also in the group of inactive carriers
- ✓ These markers could be used for
 - improved patients characterization
 - selection of candidates for HBV cure research
 - analyze the underlying pathogenetic mechanisms

Aknowledgments

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