Characterization of Resistance-Associated Variants at baseline and failure of All-Oral Antiviral Therapy of Hepatitis C

Abs # 0_12

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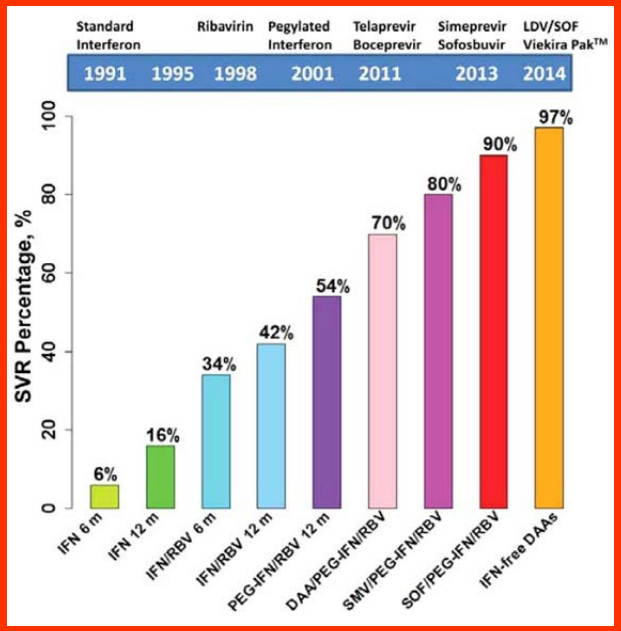
Background

✓ **Direct-acting antiviral agents (DAAs) have become the new standard of anti-HCV therapy. In spite of the very high cure rates with DAA, 1-15% of patients still fail to eliminate infection.**



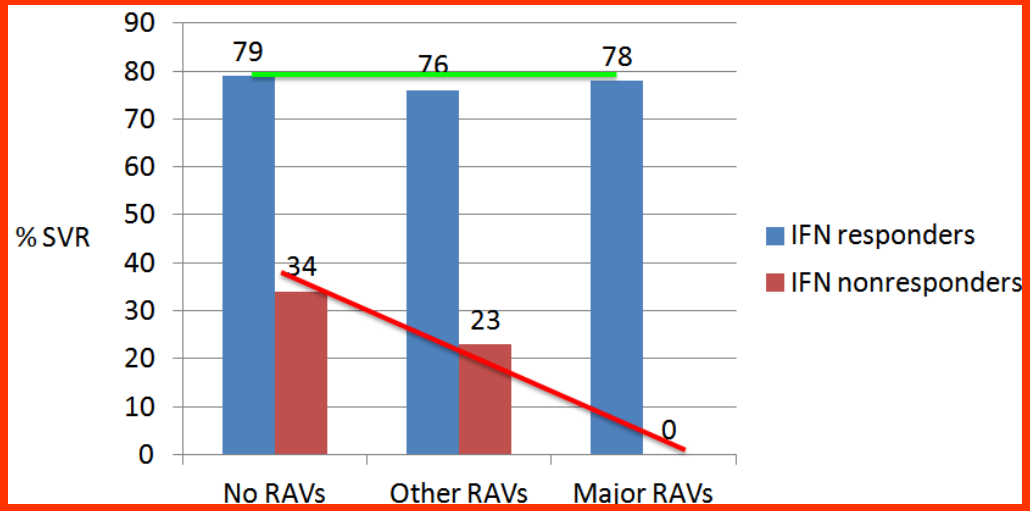
✓ **Factors that influence the ability of infected patients to be cured include:**

- **individual DAA metabolism**
- **drug interactions**
- **patient's genetic background (IL28B gene polymorphisms)**
- **presence of extensive fibrosis or cirrhosis**
- **adherence to therapy**
- **HCV resistance to DAAs.**

Background

✓ **In the context of the extremely HCV variability, information about resistance associated substitutions (RAS) prevalence at baseline is heterogeneous, partly biased by the technique used (lack of standardization, dependency on primers, reaction conditions, etc) and often incomplete.**

✓ **HCV resistance testing prior to first-line therapy is not required (EASL 2015). Indeed, the presence of pre-existing RAS as detected by population sequencing does not have a major impact on the results of therapy and will not influence the treatment decision in patients who responded to interferon (with the exception of the effect of the Q80K substitution in patients with subtype 1a infection treated with the combination of PegIFN-α, ribavirin, and simeprevir).**



✓ **Finally, most of the information has been generated with HCV genotype 1, whereas data remains scarce for other genotypes.**

Aim of the study

- ✓ **To characterize preexistence and evolution of resistance-associated substitutions in specific genotypes *in vivo*.**

- ✓ **To define the frequency of such resistance-associated variants in subjects on directly acting antivirals at baseline and failure.**

Study population

71

HCV-infected patients who started an all-oral DAA regimen enrolled in a prospective, observational study performed at the Clinic of Infectious Diseases of the L. Sacco Hospital.

GENOTYPES DISTRIBUTION:
genotype 1: 78.9% (n=56)
genotype 3: 4.2% (n=3)
genotype 4: 16.9% (n=12)

PATIENTS with CIRRHOSIS
64.8% (n=46)

PATIENTS with HIV/HCV CIONFECTION
71.8% (n=51)

PATIENTS with PREVIOUS TREATMENT
NAÏVE: 47.9% (n=34)
PegIFN-α, ribavirin: 38% (n=27)
DAA: 14.1% (n=10)

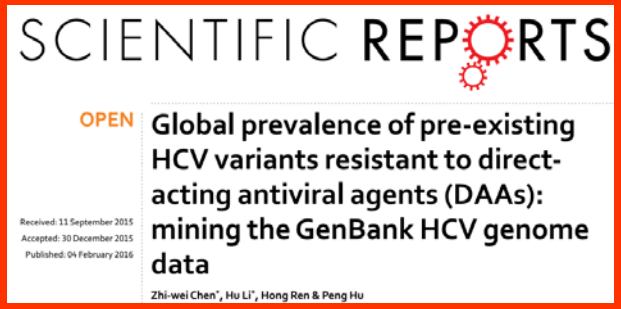
MALES
69% (n=49)

AGE
Median 52 years (range: 22-77)

	Subtype	n (%)
Genotype 1	1a	43 (60.6)
	1b	13 (18.3)
Genotype 3	3a	3 (4.2)
Genotype 4	4a	1 (1.4)
	4d	9 (12.7)
	4n	1 (1.4)
	4v	1 (1.4)

Methods

- ✓ **NS3 (aa 1-270, n=45), NS5A (aa 1-261, n=54), NS5B (aa 1-560, n=71) genes were amplified with genotype/subtype-specific protocols. Plasma samples were tested at baseline and at time of virological failure (n=6) for each subject .**
- ✓ **Genotype/subtype assignment: MEGA6.**
- ✓ **Library preparation: Nextera® XT DNA Sample Preparation and Index kit.**
- ✓ **Next generation sequencing: Illumina MiSeq platform (2 × 150 cycle paired-end).**
- ✓ **Consensus sequence: Geneious program.**
- ✓ **Mutations analysis: VirVarSeq. Minority species with frequency above 1% were considered relevant (mean coverage of 3,000).**
- ✓ **RAS were defined according to *Chen et al*. list.**
- ✓ **Statistical analysis: SPSS program.**


SCIENTIFIC REPORTS

OPEN

Global prevalence of pre-existing HCV variants resistant to direct-acting antiviral agents (DAAs): mining the GenBank HCV genome data

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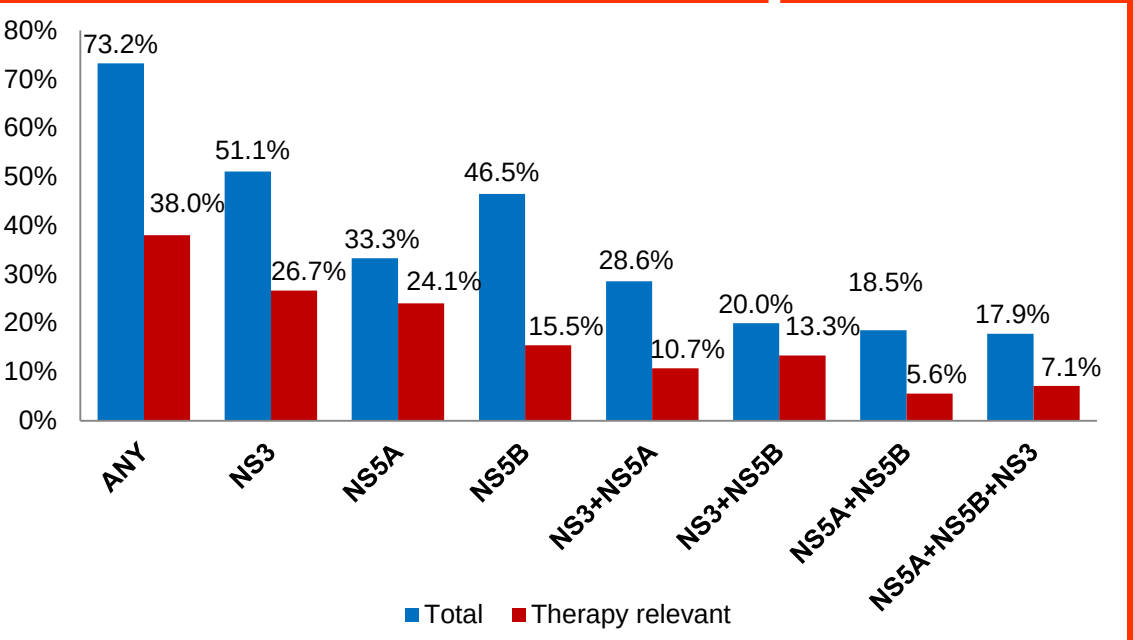
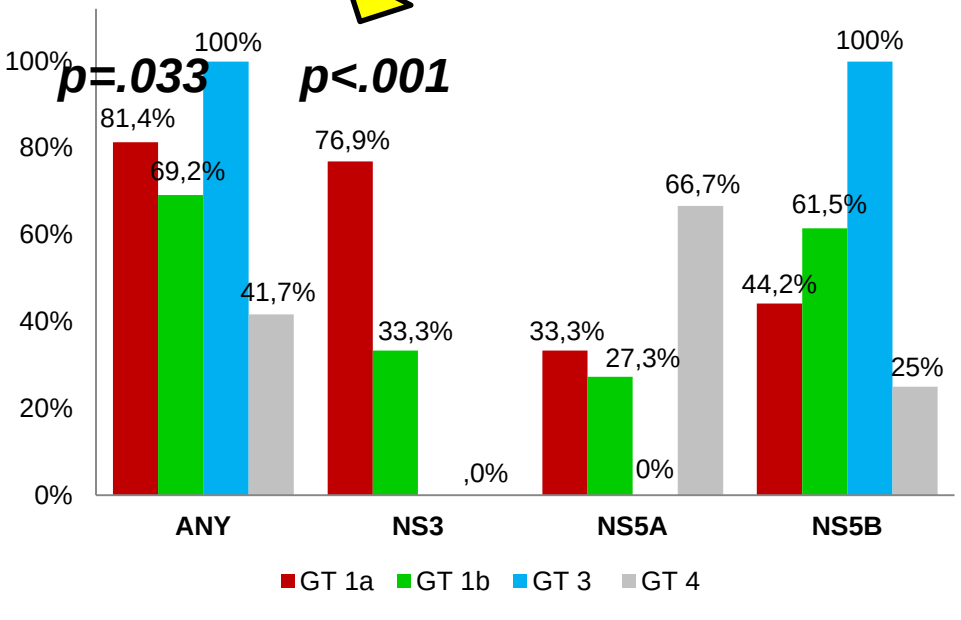
Zhi-wei Chen*, Hu Li*, Hong Ren & Peng Hu

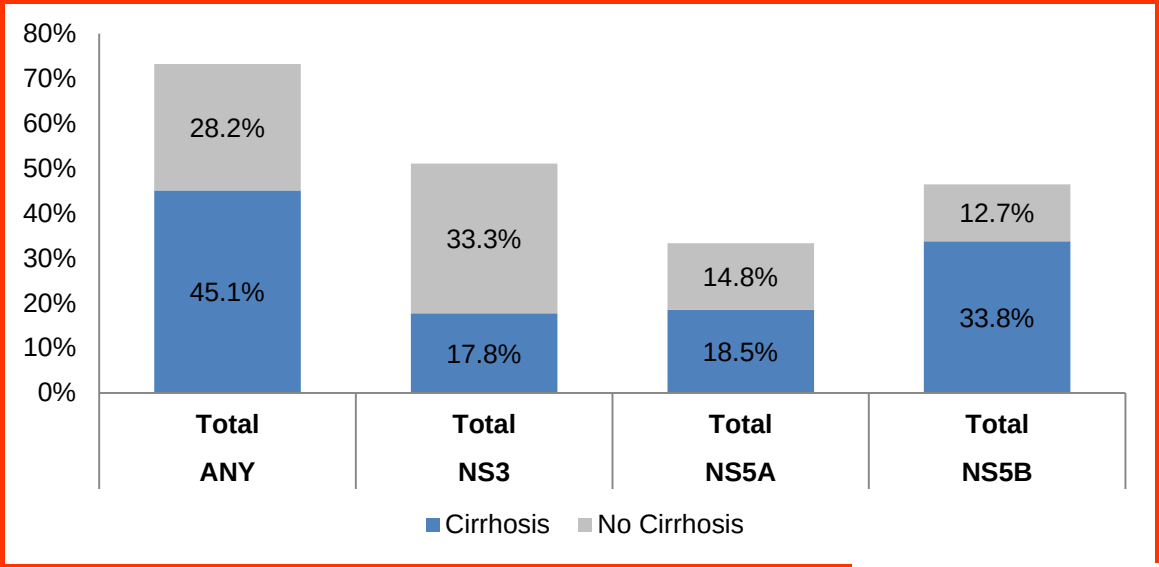
Overall prevalence of RAS

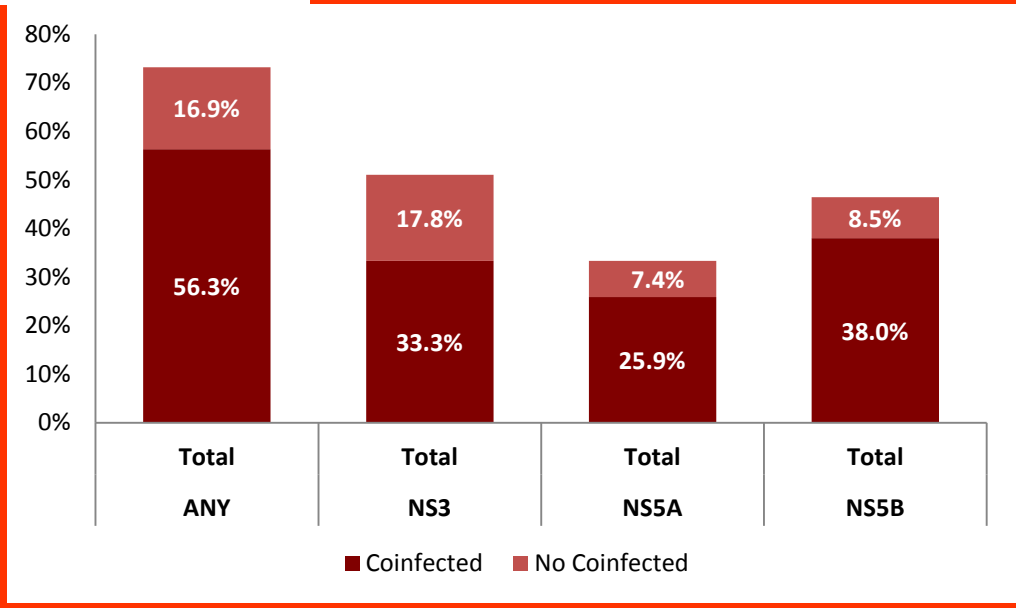


23.1%
Q80K

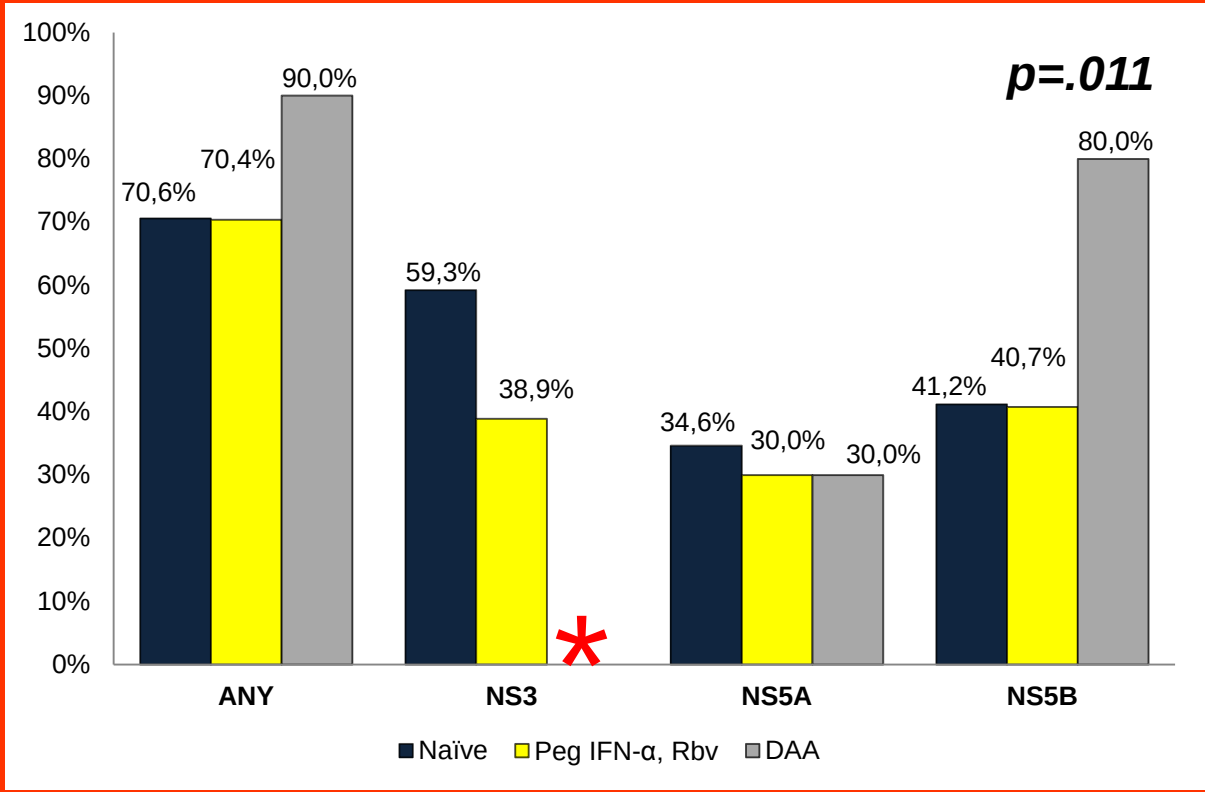


Prevalence of RAS in cirrhotic and coinfected subjects





Prevalence of RAS in naïve *vs* experienced patients



Patients failing treatment

Six patients did not achieved sustained virological response (8.4%).

							NS3		NS5A			NS5B	
		Subtypes	**Therapy**	**Cirrhosis**	**HIV Coinfection**	**Previous treatment**	SIM	PTV	DCV	LDV	OMV	SOF	DSV
1	Baseline	1a	SOF+ SMV+RBV	Yes	Yes	Naive	**Q80K**						
	Failure						**Q80K** **R155K** **D168E**						
2	Baseline	1b	PTV+OMV+DSV	No	Yes	Naive		V36A			**Y93H**		E446Q S556G D559G
	Failure							**Y56H** **D168V**			**Y93H**		E446Q S556G
3	Baseline	4d	SOF+SMV+RBV	No	Yes	PegIFN-α+RBV							
	Failure												
4	Baseline	4d	SOF+SMV+RBV	Yes	Yes	PegIFN-α+RBV							
	Failure												
*5	Baseline	4d	SOF+SMV+RBV	Yes	Yes	Naive							
	Failure												
6	Baseline	1a	PTV+OMV+DSV+RBV	Yes	No	PegIFN-α+RBV		V36G R155K			M28V		
	Failure							/			/		

Paritaprevir	PTV
Ombitasvir	OMV
Dasabuvir	DSV

Simeprevir	SMV
Daclatasvir	DCV
Ledipasvir	LDV
Sofosbuvir	SOF
Ribavirin	RBV

Conclusions

- ✓ **This study shows that RAS to NS3 and NS5B inhibitors are common and may occur at higher frequency than that reported by previous studies.**
- ✓ **The role of HCV baseline resistance mutations in the prediction of virological failure of distinct genotypes needs further investigations, in particular for genotype 4.**
- ✓ **Almost half of patients failing treatment had RAS at baseline, but these led to treatment failure only in few cases, as for Q80K in NS3 and Y93H in NS5A.**
- ✓ **No associations could be observed with cirrhosis, coinfection and previous treatments.**
- ✓ **Our data reinforce the need of genotyping at baseline for patients who will undergo to DAA as RAS are common in subtype 1a. However, a limited role of NGS at baseline is suggested in this study, since it seems not to predict selection for RAS emerging at failure.**

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