Molecular epidemiology of viral hepatitis C in Portugal

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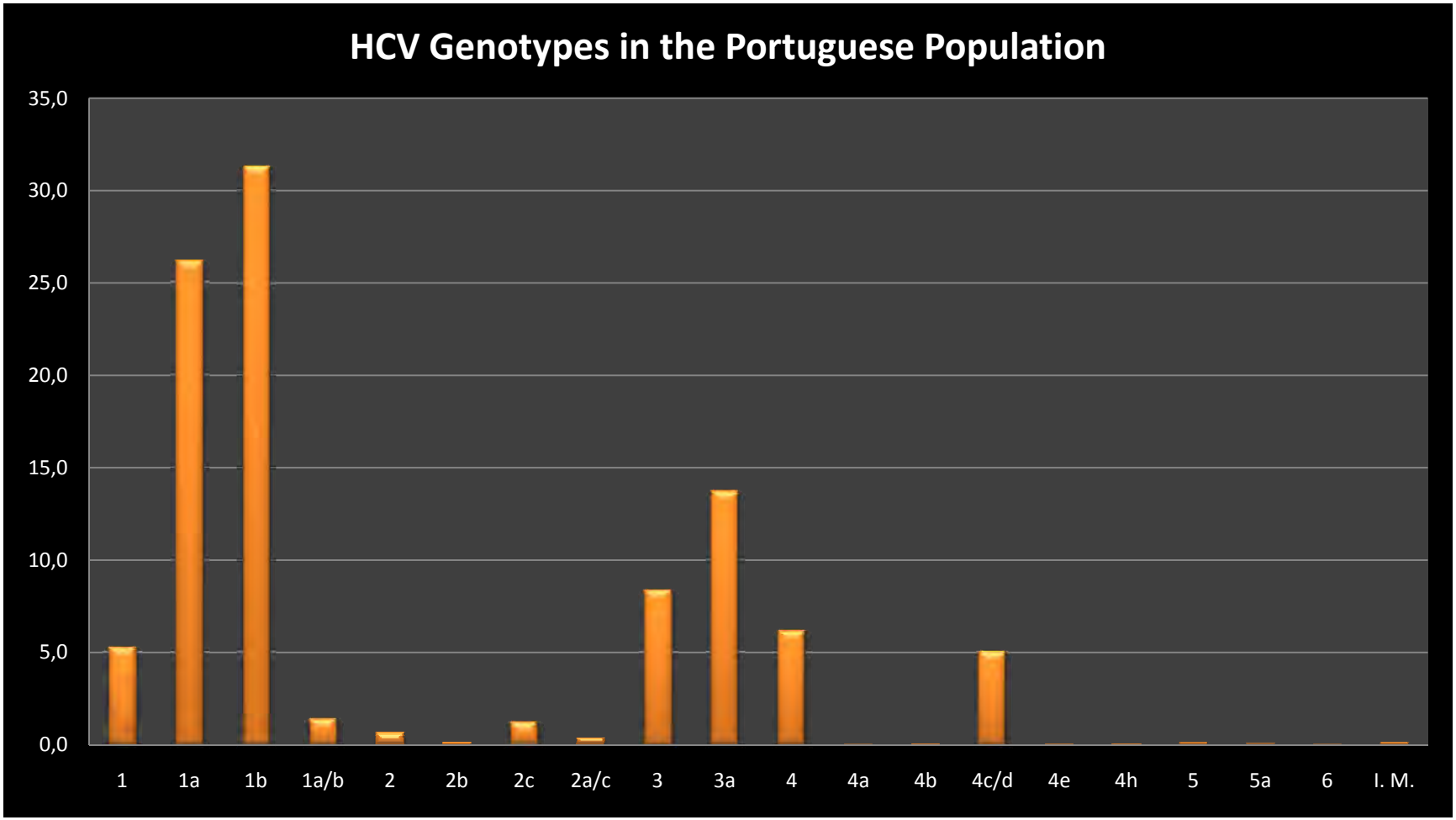
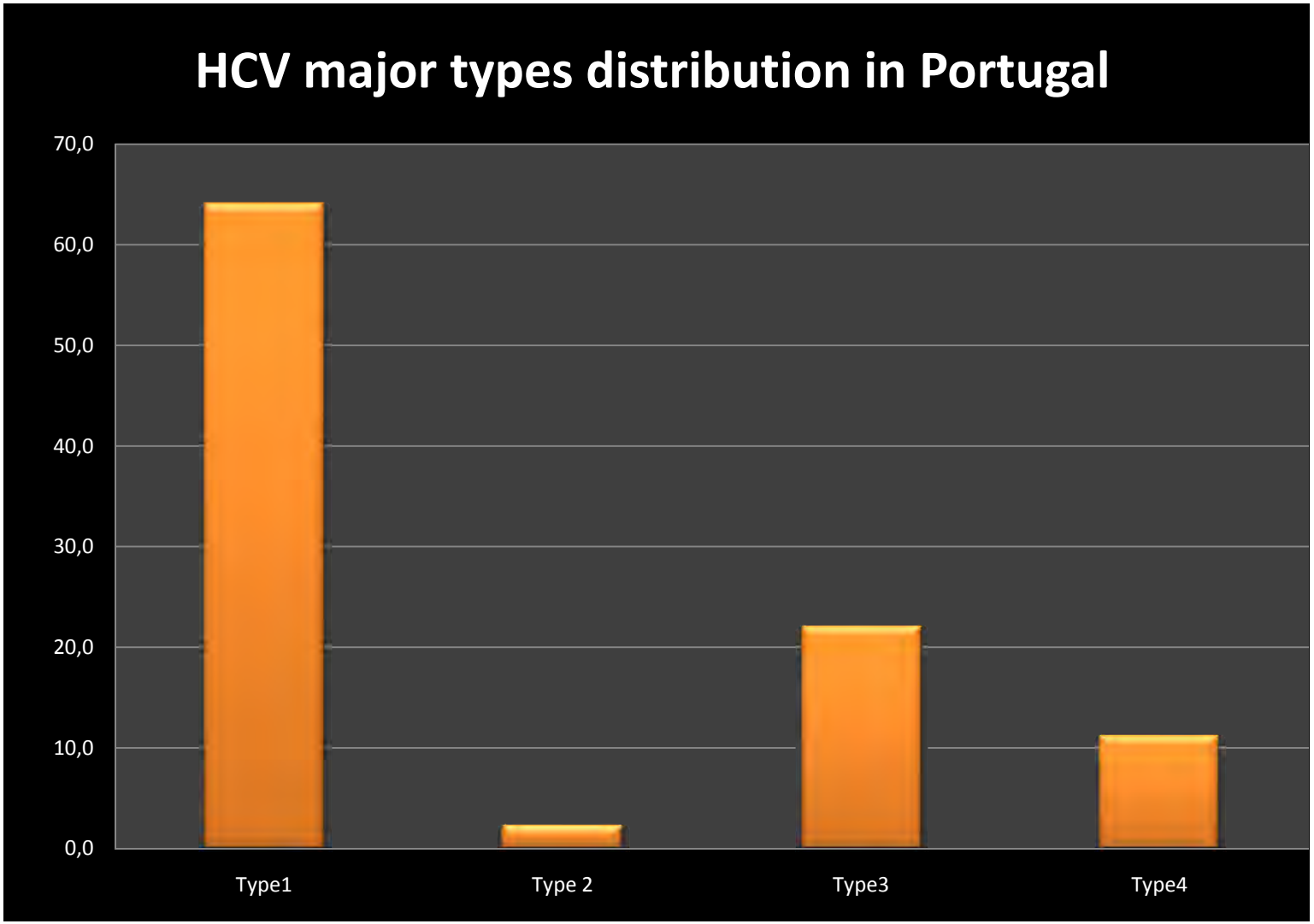
Centro de Histocompatibilidade do Centro
VHPB Meeting, Lisbon November 18-19, 2010



N= 6.243 patients

(Celene Sargento-unpb. data)



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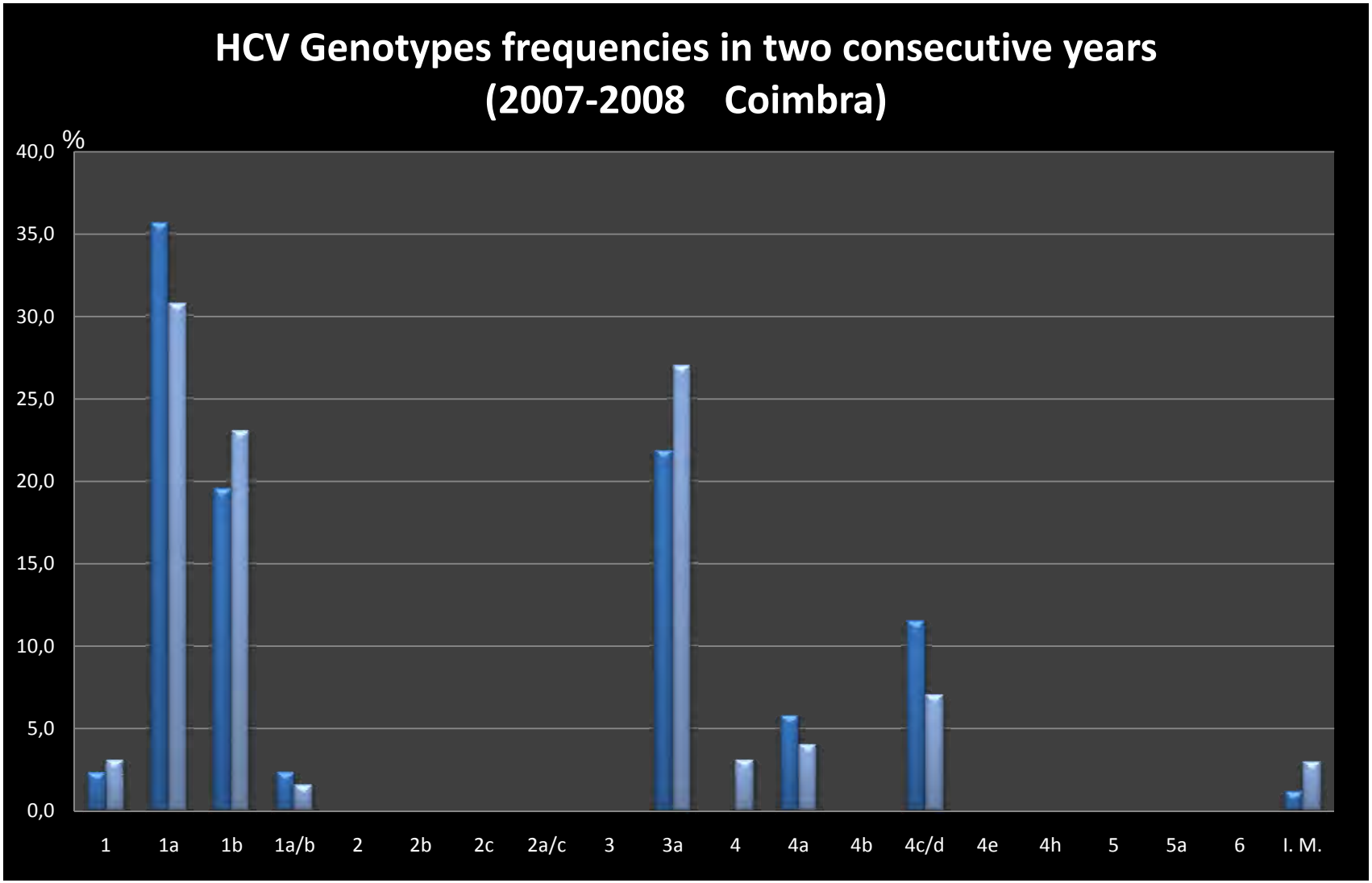

HCV Genotypes frequencies in two consecutive years
(2007-2008 Coimbra)
%
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
1
1a
1b
1a/b
2
2b
2c
2a/c
3
3a
4
4a
4b
4c/d
4e
4h
5
5a
6
I. M.

Population

(N=6243 patients from all country, including Açores and Madeira)

Gender

30% Female
70% Male

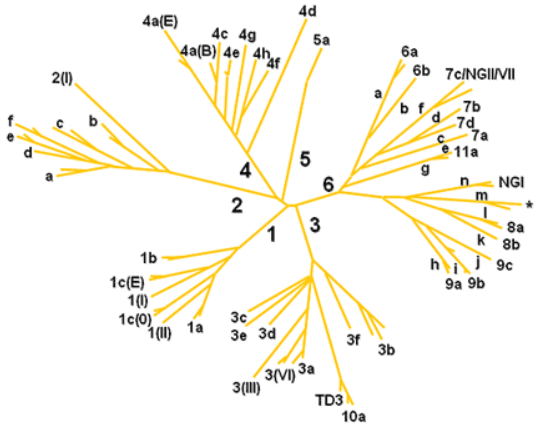
Sustained Viral Response (SVR)

Type 1 50% of S.V.R
Type 3 80%

61% overall S.V.R.

Factors afecting Sustained Viral Response

Predicting non-response to HCV infection:



HOST Factors:

- Age > 40 years
- Male gender
- Degrees of liver fibrosis
- Body mass index

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VIRAL Factors

- genotype 1
- lack of diversity in core and NS5a
- High pre treatment HCV levels

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Look in particular to the molecular variability of specific regions of HCV genome.
This is an ongoing study, started in 2004 …

Viral Factors:
First attempt to study the HCV variability ….

	Responders (n=12)	Non-Responders (n=11)
Age	*41± 11*	*40± 10*
Sex (F/M)	*3/9*	*3/8*
Genotype		
1a/b 2/4.2/5	*6*	*7*
2	*3*	*2*
3a	*3*	*2*
4	*0*	*1*
Viral load (UI/ml)	*6± 0,2*	*6.3± 0,2*
ALT (30-65)	*69± 37*	*99± 36*
AST (15-37)	*40± 18*	*56± 26*

Histology also considered but homogeneous among patients

✓ HCV Genetic region elected:

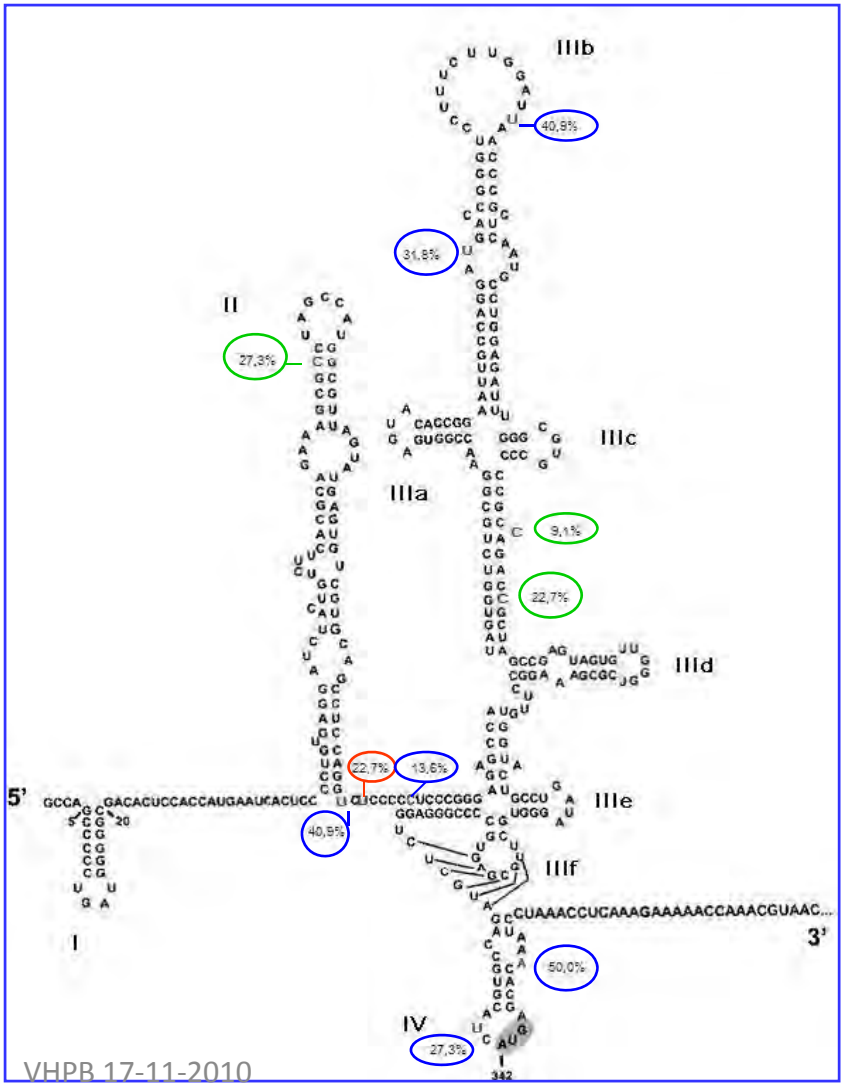
5'UTR (IRES) - conserved sequence

- Start Codon: (nt 342; *stem-loop IV*)
- *Loop IIId* - GGG Triplet (nt 266-268): it is for the interaction of IRES with the subunit 40S of the ribosom.

Mutations in the IRES could affected the efficacy of HCV replication and translation!!

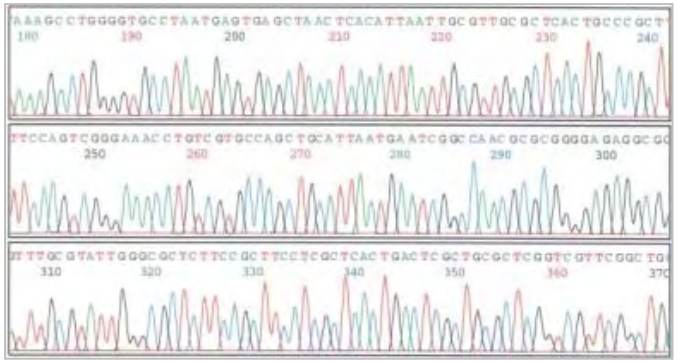
✓ Unfortunatly we didn't find significative differencies between responders and non responders

Genetic variability of 5'UTR (IRES)



Baseado em Honda M. *et al.* J. Virol. 1999

- No mutations in start codon AUG are detected among patients
- No mutations on *loop IIId*
- Couldn't identify a mutational profile related with genotypes
- Just in one patient with HCV clearance a "new" mutation was identified.



Thelu M. A., Drouet E., Hilleret M. N. & J. P. Zarski (2004). Lack of Clinical Significance of variability in the Internal Ribosome Entry Site of Hepatitis C Vírus. Journal of Medical Virology 72: 396-405

Genetic variability of core region of HCV

✓ Core region still is a conserved region of HCV genome

- Is associated with the viral presistence of the infection
- Mutations in this region could interfere in the recognition of Citotoxic T Ly

Target core epitop: (DLMGYIPAV)

Mutations in the core sequence could affect the ability of HCV to establish a persitent infection

Genetic variability of core region of HCV

- Mutations detected are predominatemente silent mutations.
- In the non responders group the number of non-sinonimous mutations (aminoacid alteration in the protein) was slightly superior
- No mutational patern was found
- No mutations in the CTL target epitop were found (DLMGYIPAV (132-140))

C terminal mutation rate of E2 protein, Genotype and Alcohol Consumption .

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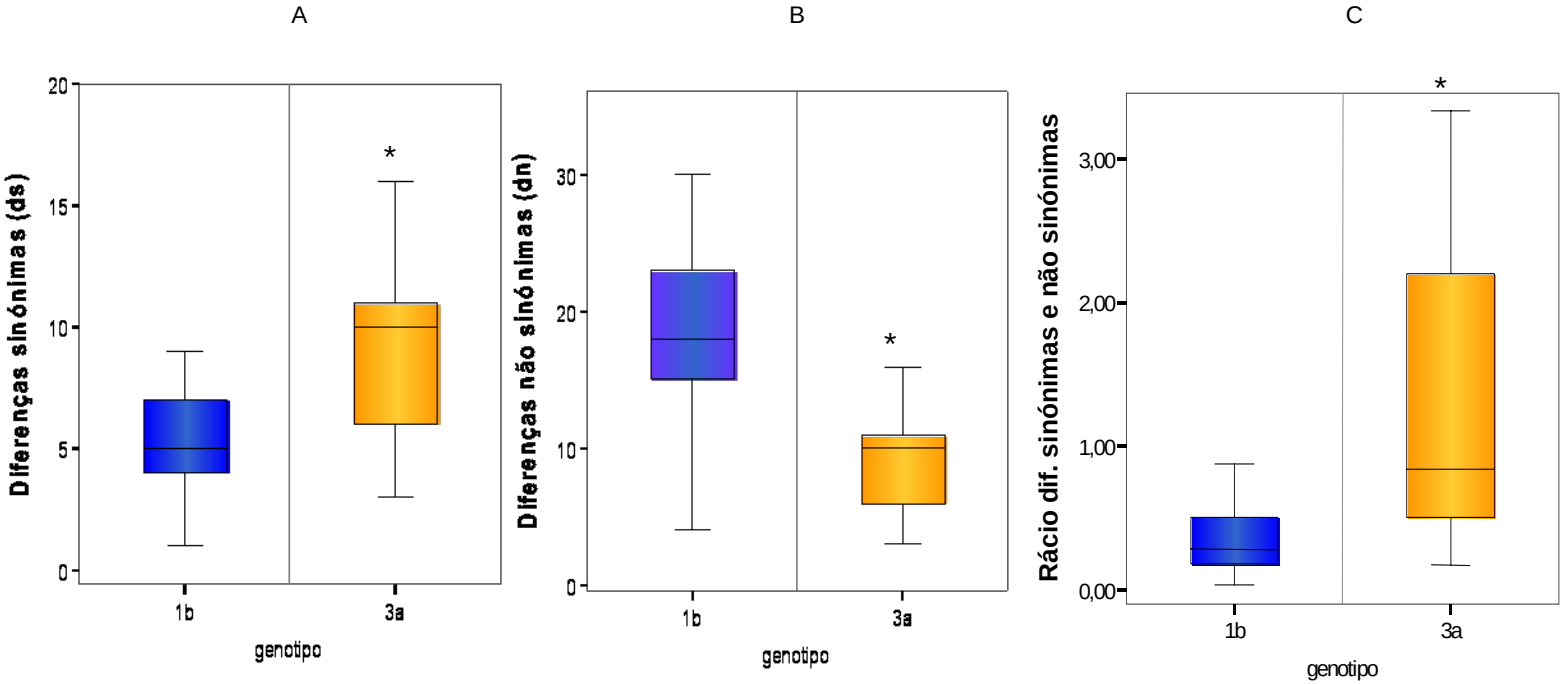
Population

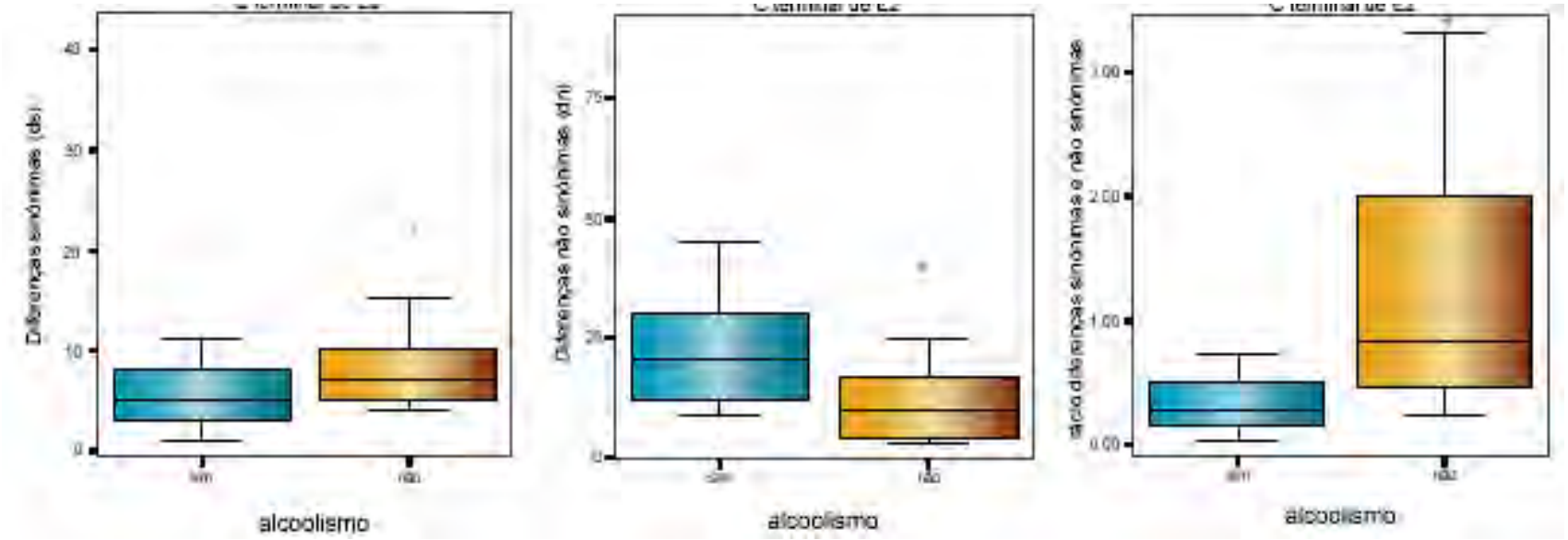
N=36	Alcohol	Non- alcohol
	N=16	N=20
Genotype 1b	N=7	N=12
Genotype 3a	N=9	N=8

C -Terminal E2 Region



- Great variability of genotype 3a (silent mutations)
- Variability of genotype 1b at aminoacid mutation level

C -terminal E2 Region: more variability in alcoholic patients



- NO EVIDENCE OF GENOTYPE MUTATIONAL PATTERN

Host Factors 1

❖ Gene expression in immune response:

stablish differences between responders and non-responders

✓Gene expression by Real Time PCR

✓Immunologic card: to study simoultaneously 92 genes involved in host immune response.

GENES EVALUATED

ACE
AGTR1
AGTR2
BAX
BCL2
BCL-XL
C3
CCR2
CCR4
CCR5
CCR7
COL4A5
COX-2
CSF-1
CSF-2
CSF-3
CXCR3
CYP1A2
CYP7A1

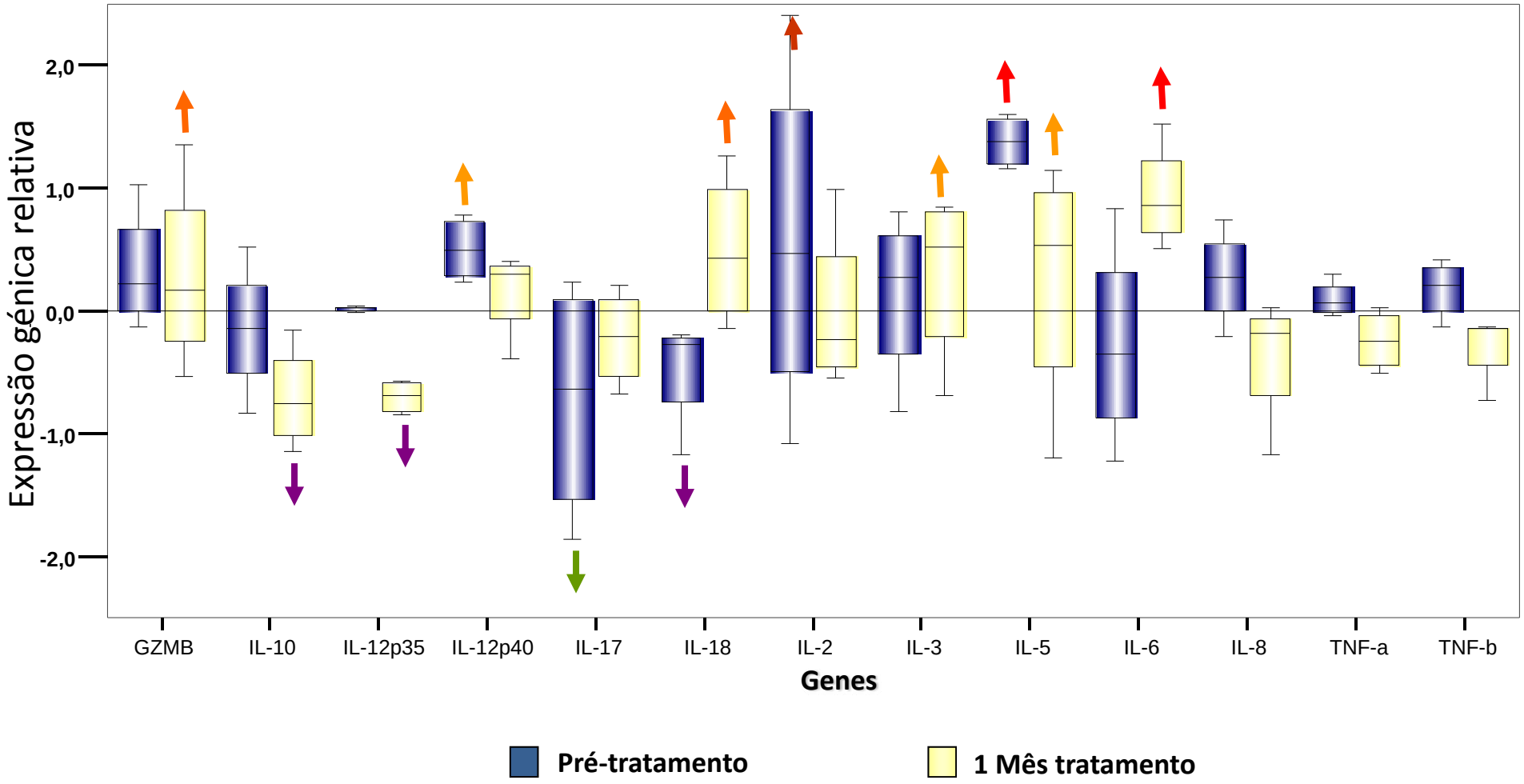
CD152
CD154
CD19
CD25
CD28
CD34
CD38
CD3
CD40
CD45
CD4
CD54
CD62E
CD68
CD71
CD80
CD86
CD8

ECE-1
EDN1
Fas
FasL
FN precursor
TNFRSF18
GNLY
GZMB
HLADRB1
HLA-DRA
HO-1
ICOS
VEGF
IkB2
TNF-b
IFN-g

NOS2
IP10
ITAC
MADH-3
MADH-7
MCP-1
MEGALIN
Mip-1a
Mip-3c
MYH6
NFKB2
PRF1
Rantes
REN
RPL3L
SKI
Stat3
TBX21

IL-10
IL-12p35
IL-12p40
IL-13
IL-15
IL-17
IL-18
IL-1a
IL-1b
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
TGF-b
TNF-a

RESULTS


Expressão génica relativa
2,0
1,0
0,0
-1,0
-2,0
GZMB
IL-10
IL-12p35
IL-12p40
IL-17
IL-18
IL-2
IL-3
IL-5
IL-6
IL-8
TNF-a
TNF-b
Genes
Pré-tratamento
1 Mês tratamento

CONCLUSIONS

Before

↑ IL-12p40, IL-2, IL-5, FAS, FASL, HLADRB1, GZMB, MADH3

↓ IL6, IL-17, IL-18, CD4

1 month after treatment

↑ IL-3, IL-6, IL-18, CD4, C3, CCR4, cox-2, HLA-DRB1, IKB2, MADH3

↓ IL-10, IL-12p35, IP-10, ITAC, MCP-1, MIP-1a

The response to treatment seams to be related with gene expression differences of effector genes. However no solid pattern was obtained between responders and non-responders (just 4 patients of each group were studied)

HOST Factors 2

Goals

Study of specific immune response anti-HCV during PegIFN + Ribavirine treatment

- ☞ Evaluation of T CD8 Lymphocytes directed against HCV proteins :
  - the frequency,
  - phenotype
  - functional caracteristics

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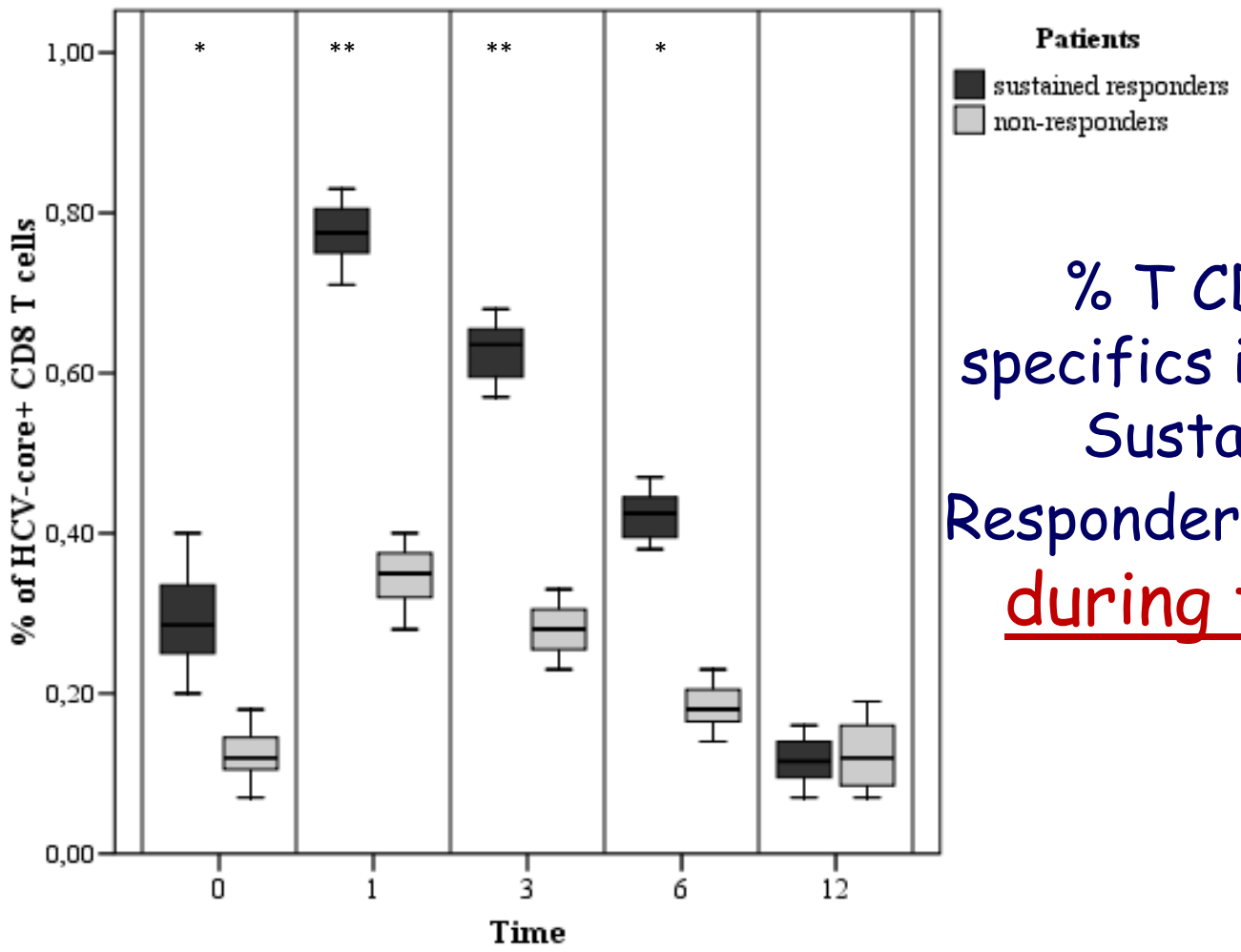

% T CD8 Ly HCV specifics is increased in Sustained Viral Responders before and during treatment.

CONCLUSION:

SUSTAINED RESPONSE IS RELATED WITH THE FREQUENCY OF CD8 T LY SPECIFIC FOR HEPATITIS C VIRUS.

STUDY PROGRESS

HOST FACTORS:

- ✓ increase the numbers (cutoff?? – this is a prospective study)
- ✓behaviour of dendritic cells (DCs)
- ✓IL28B polymorphism (we have started with normal population).

VIRAL FACTORS:

- ✓ predicting protein conformation of hypervariable regions (HVR1 and HVR2 ?!)
- ✓revisiting the core region
- ✓mutational pattern in NS5A and NS3 genes

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- Fátima Simões
- Angela Moreira
- Andreia Santos

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Hospitais da Universidade de Coimbra

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and many others

Centro Hospitalar de Coimbra

Dr. Cristina Valente

Enf. Beatriz

and many others

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Universidade de Coimbra

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All the staff of Centro de Histocompatibilidade do Centro

THANK YOU!

QUANTIFICAÇÃO E CARACTERIZAÇÃO FENOTÍPICA DE CTL