IL28B variants association with sustained virologic response and plasma cytokine levels in HIV/HCV coinfected patients

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- Polymorphisms within and near to *IL28B* gene predict hepatitis C virus (HCV) treatment (peg-IFN/RBV) success in either HCV mono and HIV/HCV coinfected patients.
- The first two SNPs identified by GWAS were rs12979860 and rs8099917, and therefore they have been deeply studied.
- Although these SNPs are both excellent predictors of HCV treatment success, the true causal mutation has not been identified yet, and for this reason new SNPs need to be explored.
- In total, 20 polymorphisms have been investigated in relation to SVR, but only eight of them have been more studied (mainly in HCV patients).

However, few data have been published in coinfected for other relevant *IL28B* SNPs such as rs12980275 (upstream), rs11881222 (intron 3) and rs7248668 (downstream).
-To estimate the impact of four different *IL28B* polymorphisms on sustained virologic response in HIV/HCV patients (rs12980275, rs8099917, rs7248668, and rs11881222)

-To investigate whether haplotypes or the combination between these polymorphisms improve HCV treatment success prediction.
MATERIAL AND METHODS

-324 HIV/HCV coinfected patients: HGUGM & HCIII
-All patients started treatment with peg-IFN/RBV therapy

-Sustained virologic response (SVR) to HCV treatment was considered as undetectable plasma HCV viral load six months after treatment cessation.

<table>
<thead>
<tr>
<th>Characteristic of patients</th>
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<tbody>
<tr>
<td>No.</td>
<td>324</td>
</tr>
<tr>
<td>Male *</td>
<td>249 (76.9%)</td>
</tr>
<tr>
<td>Age (years) †</td>
<td>41.9 (38.6 – 45.2)</td>
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<tr>
<td>IVDU *</td>
<td>284 (87.7%)</td>
</tr>
<tr>
<td>HAART *</td>
<td>274 (84.6%)</td>
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</tbody>
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Anthropometric values
- Height (m) †: 1.70 (1.65 – 1.75)
- Weight (Kgr) †: 67 (60 - 76)
- BMI (kg/m²) †: 23.1 (21.2 – 25.4)
- BMI ≥25 kg/m²: 92 (29.6%)

HIV markers
- Nadir CD4+ T-cells/μL †: 224 (131 - 330)
- Nadir CD4+ <200 cells/μL *: 141 (43.5%)
- Baseline CD4+ T-cells/μL †: 468 (372 - 672)
- CD4+ ≥500 cells/μL *: 145 (44.8%)
- HIV-RNA <50 copies/mL *: 212 (65.4%)

HCV markers *
- HCV-genotype
  - 1: 184 (56.8%)
  - 3: 103 (31.8%)
  - 4: 37 (11.4%)
- Baseline HCV-RNA ≥500,000 IU/mL: 235 (72.5%)
- Baseline Log₁₀ HCV-RNA (IU/mL) †: 6.09 (5.62 – 6.74)

Liver fibrosis (n= 289)*
- Significant fibrosis (F≥2): 180 (62.3%)
- Advanced fibrosis (F≥3): 98 (33.6%)
- Cirrhosis (F4): 51 (17.6%)
-Four *IL28B* SNPs were genotyped by GoldenGate® assay with VeraCode technology (Illumina)

rs12980275
rs11881222
rs8099917
rs7248668
RESULTS

Pairwise linkage disequilibrium (LD) patterns for four the polymorphisms. Each diagonal represents a different SNP, with each square representing a pairwise comparison between two SNPs.

Due to the high LD among *IL28B* polymorphisms, we obtained the same or highly similar results for rs12980275/rs11881222 and rs8099917/rs7248668 couples.

In order to avoid redundancy, only show results for rs12980275 and rs8099917 are shown.
We performed multivariate logistic regression analysis to investigate the association between favorable \( IL28B \) genotypes and SVR.

**RESULTS**

Statistically significant differences are shown in black.

(a), Adjusted by HCV-genotype 1 or 4 versus 3, HCV-RNA \( \geq 500,000 \) IU/mL and significant fibrosis (F\( \geq 2 \)).

(b), Adjusted by HCV-RNA \( \geq 500,000 \) IU/ml and significant fibrosis (F\( \geq 2 \)).

haplotypes formed by rs12980275, rs11881222, rs8099917, rs7248668.
RESULTS

However, the use of a decision tree tool appeared to improve treatment success prediction in genotype 1/4 patients. Nodes have been stratified by *IL28B* genotypes for rs12980275 and rs8099917 polymorphisms.

For rs12980275:
- Unfavorable (AG + GG): 134 Patients, 30.6% SVR
- Favorable (AA): 222 Patients, 41.9% SVR
  - Significance: p < 0.001

For rs8099917:
- Unfavorable (GT + GG): 92 Patients, 23.9% SVR
- Favorable (TT): 88 Patients, 59.1% SVR
  - Significance: p = 0.014
The cytokine profile also plays an important role in treatment outcome and it seems to modulate the immune response against HCV.

However, scarce data have been published on plasma levels of cytokines with *IL28B* in HIV/HCV-coinfected patients.
-13 cytokines were assessed in plasma samples (Human Th1/Th2/Th9/Th17/Th22 FlowCytomix Multiplex): IFN-gamma, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p70, IL-13, IL-17A, IL-22 and TNF-alpha

To explore the behaviour of plasma cytokine levels in these patients

OBJECTIVES

MATERIAL AND METHODS
Cytokine levels of 57 HIV/HCV coinfected patients at pretreatment, according to *IL28B* (rs12980275) genotype and sustained virologic response (SVR)
CONCLUSIONS

-The four *IL28B* polymorphisms tested alone or by haplotypes showed a significant association with HCV clearance after HCV therapy.

-Although haplotypes did not improve the prediction of SVR, the decision tree was very useful for improving the prediction of HCV treatment success in the most difficult-to-treat patients.

-The cytokine environment was much more favorable in patients with favourable genotype for *IL28B* (rs12980275 (AA)) who achieved SVR.
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