



## Role of *IL28b* Polymorphisms on the Response to Treatment with Pegylated Interferon Alpha-2a and Ribavirin in Hcv-Infected Patients after Liver Transplantation

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### ABSTRACT

**BACKGROUND:** Many studies have provided evidence of the role of *IL28B* variants in the outcome of chronic HCV infection, but there is much less information regarding the influence of these variants in the post liver transplantation treatment with conflicting results. The aim of this study was to contribute to clarify the role of the polymorphism in rs12979860 in the pair recipient/donor in the response to the treatment with IFN $\alpha$  and RBV in a cohort of patients undergoing liver transplantation.

**METHODS:** A total of 69 pairs of patients and donors were included in the study. The genotyping of the SNP of *IL28B*, rs12979860, was performed using a TaqMan allelic discrimination assay. In order to minimize the impact of chimerism, a set of 13 short tandem repeat

(STR) markers was used when the follow-up biopsy was used to genotype donor.

**RESULTS:** Regarding the rates of end of treatment response (ETR), the receptor/donor combinations CC/CC and CC/non-CC or non-CC/CC had the same values 73.3% and 80.6%, respectively) and only the combination non-CC/non-CC (40.0%) had a significantly lower value ( $p=0.005$ ; OR: 5.4, 95% CI, 1.22-22.9). Similarly, in the analysis of sustained virological response (SVR), the same trend was observed, only the combination non-CC/non-CC had a poorer SVR rate (40.0%, vs. 66.7% in CC/CC and 64.5% in CC/non-CC or non-CC/CC,  $p=0.08$ ; OR: 2.81, 95% CI, 0.73-11.06)

**CONCLUSION:** Our results suggest that the response to the antiviral therapy post liver transplantation is associated to the *IL28B* genotype of both, the recipient and the donor.

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**Key Words:** Hepatitis C virus; *IL-28B*; Interferon alpha-2a; Liver transplant; Polymorphism

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### Abbreviations

ETR: end of treatment response; HCV: hepatitis C virus; HIV: human immune deficiency virus; IFN- $\alpha$ : interferon- $\alpha$ ; IFN- $\lambda$ 3: type III IFN; *IL28B*: interleukin-28B; LT: liver transplantation; PBMC:



peripheral blood mononuclear cells; RBV: ribavirin; RVR: rapid virologic response; SNPs: single nucleotide polymorphisms; STR: short tandem repeat; SVR: sustained virological response.

## INTRODUCTION

The hepatitis C virus (HCV) infection is a major global health problem affecting about 180 million people worldwide. This infection results in a chronic active hepatitis in more than 80% of the infected patients, of which 20%-30% develop progressive fibrosis and cirrhosis. This infection is the leading cause of end-stage liver disease necessitating liver transplantation (LT) in developed countries<sup>[1]</sup>. Without treatment, most of the grafts are infected *de novo*, nevertheless, this problem can be mitigated by the achievement of a sustained virological response (SVR) using the combined therapy of interferon- $\alpha$  (IFN $\alpha$ ) and ribavirin (RBV). This standard treatment does not produce sustained response in all the patients and there are different factors that may influence this response. Thus, in addition to the role of several viral factors in the outcome of the disease, increasing evidences support the importance of host factors in the success or failure of the antiviral therapy<sup>[2,3]</sup>. Recently, three independent genome-wide association studies performed in HCV infected patients have identified a region located on chromosome 19q13, around the interleukin-28B (*IL28B*) gene, as associated with the response to treatment. Specifically, the variants associated were two single nucleotide polymorphisms (SNPs), the rs12979860, located 3 kilobases (kb) upstream of the *IL28B* gene and, the rs8099917, situated in the intergenic region between *IL28B* and *IL28A*. These variants have been described as associated with SVR in patients chronically infected with HCV genotype 1, treated with IFN $\alpha$  and RBV<sup>[4-6]</sup>. At the present, it is well established that the rs12979860CC genotype is associated with both, higher rates of spontaneous resolution of HCV infection as well as SVR (compared with CT/TT genotypes). In the same way, these variants have also been studied in order to investigate their relationship with the progression and response to treatment after LT. Intuitively, one would think that, the patterns of response to antiviral therapy after LT are equal than those observed in non-transplanted HCV infected patients. Nevertheless, some studies suggest that the sensitivity to interferon-based therapy may change after LT. Thus, some no-responders patients to the combined treatment before of LT, became responders after transplantation and vice-versa<sup>[7,8]</sup>. These changes in the pattern of the response could be related with the graft. In this sense several studies have investigated the relationship between polymorphisms in the above mentioned variants of the *IL28* region in: the recipient, the graft and the pair donor/recipient, in the response to the treatment, but with conflicting results<sup>[9,10]</sup>. The aim of this study was to contribute to clarify the role of the polymorphism in rs12979860 in the pair recipient/donor in the response to the treatment with IFN $\alpha$  and RBV in a cohort of patients undergoing liver transplantation.

## PATIENTS AND METHODS

The present study included a retrospective analysis of a cohort of 69 patients with chronic hepatitis C who underwent a liver transplant between 1996 and 2010 and who received an antiviral therapy post-transplant at the Hospital Universitario Virgen del Rocío in Seville, Spain. All patients provided a written informed consent and the study was approved by the Ethic Committee of the Hospital Universitario Virgen del Rocío in accordance with guidelines set forth the 1975 declaration of Helsinki. All the patients had anti-

HCV antibodies and HCV RNA in serum and they suffered a histologically proven recurrent hepatitis. Patients co-infected with human immune deficiency virus (HIV), hepatitis B virus and those with other liver diseases were excluded. Anti-HCV, HBsAg and anti-HIV were determined by commercially available methods (HCV 3.0 test, ORTHO, and Enzygnost hepatitis B surface antigen, 5.0 and anti-human immune deficiency virus-1/2 plus; DADE, Behring, respectively).

The hepatic disease was ranked according to Scheuer index from F0 (absence of fibrosis) to F4 (cirrhosis stage)<sup>[11]</sup>. After LT, all patients were treated with Peg-IFN $\alpha$ 2a 180  $\mu$ g/week and RBV 1000-1200 mg/day according to body weight, trying to use a full dose from the start of therapy<sup>[12-14]</sup>. The dose of RBV or Peg IFN $\alpha$ 2a was reduced and the transfusion of packed red blood cells and growth factors (erythropoietin and granulocyte colony-stimulating factor G-CSF) were used when it was considered necessary, but trying to maintain the highest possible dose of RBV and Peg IFN $\alpha$ 2a from the beginning and throughout the 4 weeks of treatment. An extension of the standard period of use of the therapy was proposed to those patients without a rapid virologic response (RVR, defined as undetectable HCV-RNA at week 4). The prolongation time was adjusted according the infective viral genotypes (48 weeks to those infected by genotypes 2-3 and 72 to patients with genotypes 1-4). End of response to the treatment (ERT) was defined as undetectable viral load at the end of therapy and sustained virological response (SVR) as HCV RNA undetectable in serum at 24 weeks after completing the antiviral therapy.

Genomic DNA of the recipients was obtained from peripheral blood mononuclear cells (PBMC) using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) when this source was available ( $n=56$ ). In those cases in which PMBC were not available, DNA was extracted from stored paraffin-fixed liver tissue blocks of liver explants (in the case of the recipients,  $n=13$ ) and grafts (all the donors) using QIAamp DNA FFPE Tissue Kit (Qiagen). Genotyping of rs12979860 was carried out using a TaqMan<sup>®</sup> allelic discrimination assay (Applied Biosystems, Barcelona, Spain) in a 7900HT Fast Real Time PCR System (Applied Biosystems). Genotyping of each sample was automatically attributed by the SDS 1.3 software for allelic discrimination. In order to minimize the impact of chimerism<sup>[15]</sup> (namely, partial contamination of the biopsy with cells from the recipient), a set of 13 short tandem repeat (STR) markers (CSF1PO, FGA, THO1, TPOX, SVA, DS31358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11) was included in the study. These markers were analyzed using an automated fluorescence analysis of PCR-amplified STR loci. The use of this set of markers named CODIS (Combined DNA Index System) permits to discard the presence of chimerism<sup>[16,17]</sup>. Those follow-up liver biopsies in which chimerism was detected were excluded from the study (Figure 1S).

Regarding statistical methods, the Fisher's exact test was used to compare categorical variables whereas the quantitative variables were analyzed using non-parametric tests (Mann-Whitney for unpaired groups and Wilcoxon for paired groups). Those two-sided p-values less than 0.05 were considered statistically significant.

## RESULTS

The overall cohort consisted of 69 patients (46 men and 23 women) with a mean age of 56 years. Fifty-eight (84.1%) patients were infected by viral genotype 1 and 47 (68.1%) had levels of HCV-RNA greater than 800.000 IU/mL. Only five (7%) patients had an acute

hepatitis whereas the rest (93%) had a chronic hepatitis, an advanced fibrosis (F3-F4) was diagnosed in 50% of this group. A total of 21 (30.4%) patients did not have response to the pre-transplant antiviral treatment. The median time between LT and the beginning of the antiviral therapy was 22.2 months (11.9-45.6). Twenty-four patients without RVR received prolonged treatment; four of them were infected by non-G1 viral genotype. Two pairs were eliminated because of low quality of the DNA or insufficient specimen. After genotyping with the CODIS panel to avoid possible chimerism in samples from follow-up liver biopsies (Figures S1), six pairs were excluded by chimerism, so a total of 61 couples were finally included. Table 1 displays some clinical and epidemiological characteristics of the patients included in the whole cohort.

The distribution of *IL28B* polymorphisms in the pairs donor-recipient are shown in Figure 1. The frequencies of genotypes CC, CT and TT of the rs12979860 in recipient samples were 42.6%, 41.0% and 16.4% respectively, while in donors the distribution was 57.4%, 31.1% and 11.5%. Therefore, the CC genotype frequency was reduced among patients while, in contrast, the frequency of CT + TT increased.

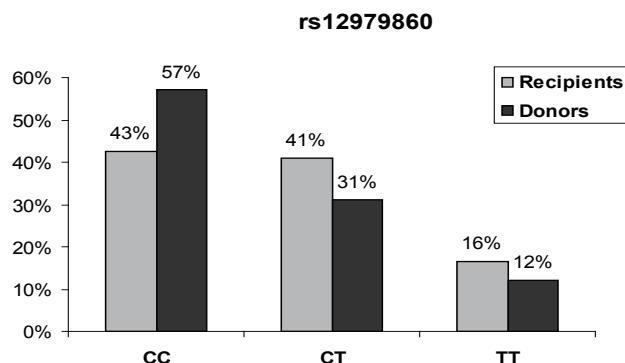
The rate of ETR and SVR were 69.1% (47/68) and 60.3% (41/68) respectively. The rate of ETR and SVR according to recipient *IL28B* genotypes were 81.5% CC, 72.4% CT and 33.3% TT and 66.7% CC, 69.0% CT and 25% TT respectively (Figure 2A) ( $p=0.015$ ; OR: 6.33, 95% CI [1.33-34.07]). Similarly, the rates of ETR and SVR according to the donor genotype were: 74.3% CC, 73.7% CT and 28.6%TT

and 65.7% CC, 57.9% CT and 28.6% TT respectively ( $p=0.18$ ; OR: 4.25, 95% CI [0.63-35.37] and (Figure 2B). To evaluate a possible synergistic effect, the rates of response to the treatment in the different combinations of donor/recipient genotypes were analyzed grouping according to the presence of zero, one or two risk genetic factors: donor and recipient CC, donor or recipient CC and donor and recipients non-CC. Fifteen recipient-donor pairs were CC/CC (zero risk factors), in others thirty-one cases the combination was CC/non-CC (CT or TT) or non-CC/CC (one risk factor), and finally fifteen pairs were non-CC/ non-CC (two risk factors). Regarding ETR, the rates of response were no significant different between the CC/CC group (73.3%) and CC/non-CC or non-CC/CC (80.6%). Nevertheless, the rate of response was significantly lower in the non-CC/non-CC group (40.0%). Therefore the combinations CC/CC, CC/non-CC and non-CC/CC, were considered as good responders and grouped to compare with the group with poorer response: non-CC/non-CC ( $p=0.005$ ; OR: 5.4, 95% CI [1.22-22.9]). Similarly, analysis of SVR revealed no significant differences between the first two groups (66.7% CC/CC vs. 64.5% in CC/non-CC or non-CC/CC) while the combination non-CC/non-CC had the lowest SVR rate (40.0%). Nevertheless, in this case, the comparison between good responders and poor responders did not reach significance, although the same trend as in the case of ETR was observed ( $p=0.08$ ; OR: 2.81, 95% CI [0.73-11.06]) (Figure 3A). It has been well established in non transplanted patients, the relationship between infection by non-G1 viral genotypes and a better SVR. In our cohort, no statistically significant differences were observed in the response post-transplantation between G1 vs. non-G1 viral genotype. However, because of 14.8% of our patients were non-G1 patients, data were reanalyzed including only patients infected by G1. The results were similar to those obtained with the complete cohort. Regarding ETR, significant differences were observed to compare good and poor responders ( $p=0.01$ ; OR: 4.66, 95% CI [1.10-20.64]) and for SVR this same trend was observed although the differences did not reach significance ( $p=0.13$ ; OR: 2.76, 95% CI [0.69-11.45]) (Figure 3B).

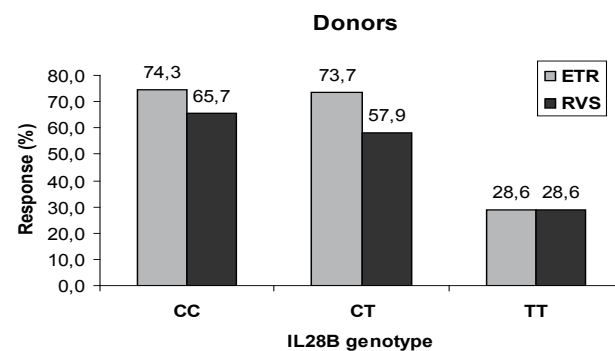
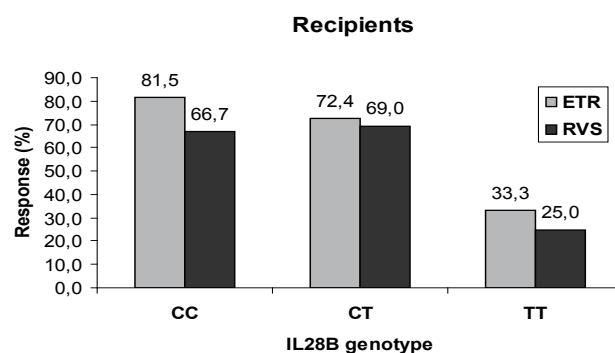
**Table 1** Epidemiological and clinical characteristics of the HCV infected LT patients included in this study.

Characteristics	Whole cohort N=69	IL-28B cohort <sup>1</sup> N=61	<i>p</i>
Gender (males)	46 (66.7)	41 (67.2)	ns
Age (years)	56 [50-64]	56 [50-63]	ns
Viral genotype 1	58 (84.1)	52 (85.2)	ns
Chronic hepatitis	64 (92.8)	57 (93.4)	ns
Rate of fibrosis (F3-F4)	32 (50.0)	27 (47.4)	ns
Bilirubin, mg/dl	1.1 [0.8-1.8]	1.1 [0.8-1.8]	ns
ALT (IU/mL)	107.0 [61.5-230.5]	115.0 [57.5-246.0]	ns
AST (IU/mL)	147.0 [58.5-317.5]	147.0 [58.5-317.5]	ns
SVR	41 (59.4)	36 (59.0)	ns
RVR	20 (29.9)	19 (32.2)	ns
Baseline VL (log <sub>10</sub> IU/mL)	5.62 [4.26-12.11]	5.62 [5.30-12.11]	ns
Duration of treatment after LT (weeks)	48 [30-71]	48 [33-71]	ns

For quantitative variables the median values and [the ranges] are shown whereas for qualitative variables the number of individuals and (%) are displayed. <sup>1</sup>This group does not include those patients from pairs with chimerism in samples from follow-up liver biopsies, low quality of the DNA or insufficient specimen.



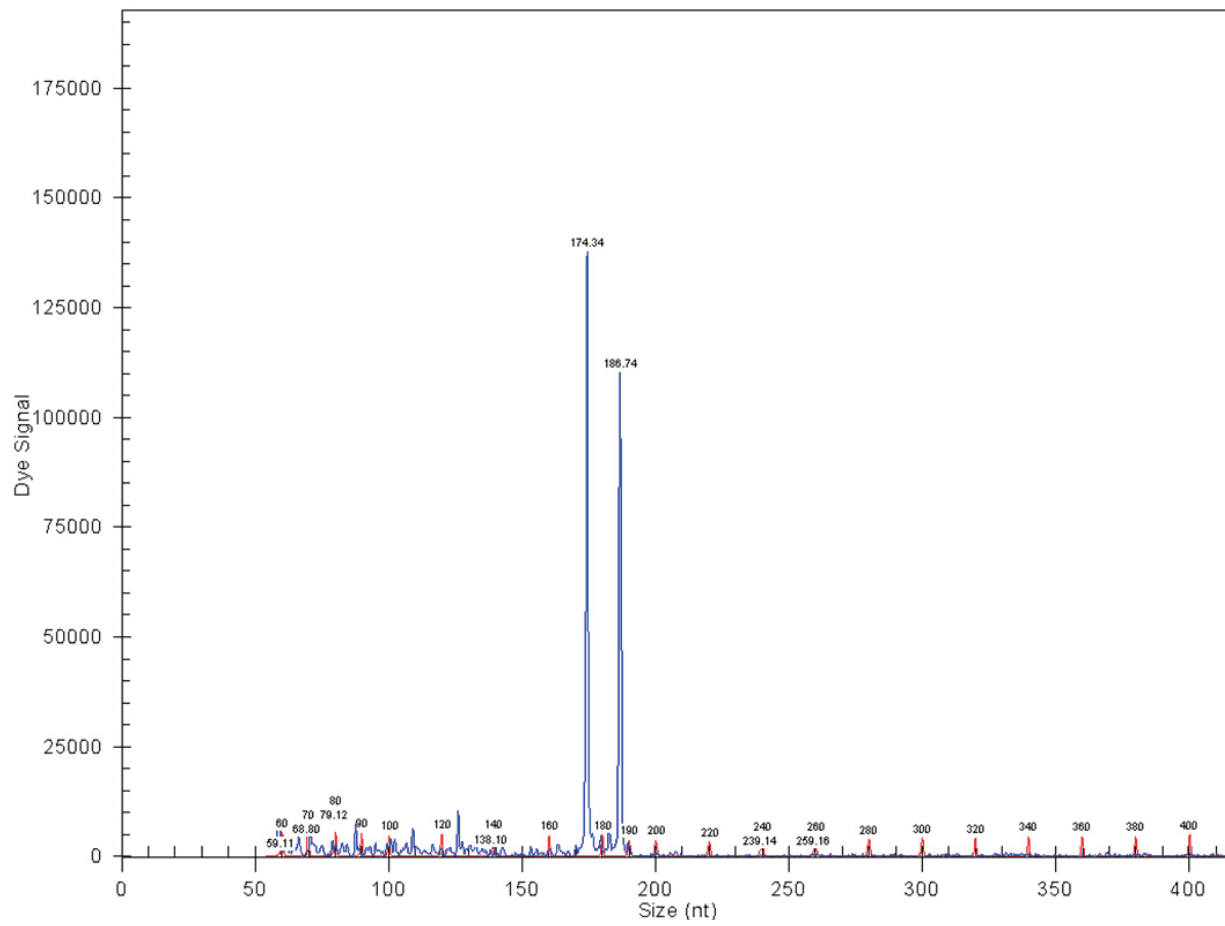
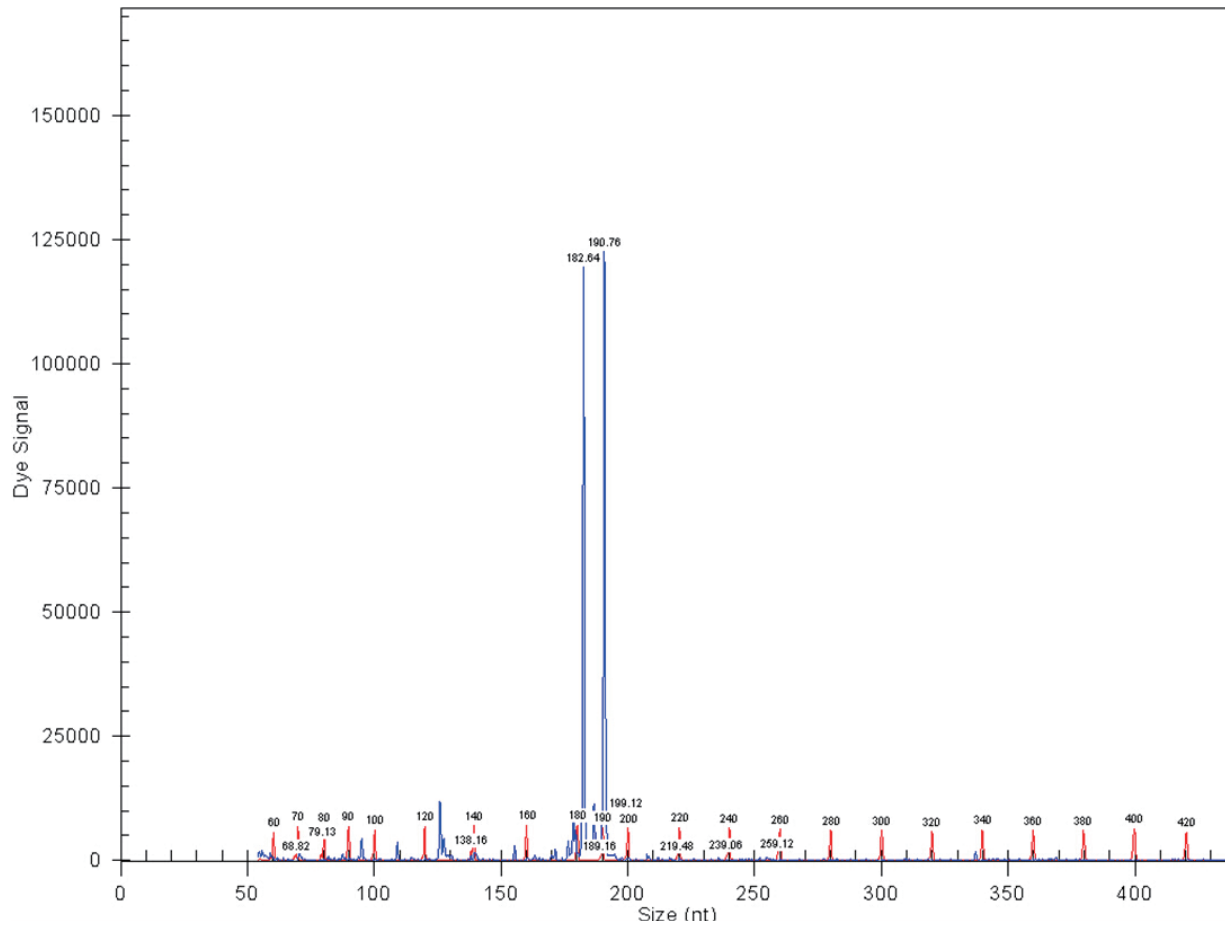
**Figure 1** Distribution of the rs12979860 variants in the recipients and donors included in this study.



**Figure 2** Rate of ETR and RVS in patients stratified according the IL28B genotype of the (A) recipients and (B) donors.

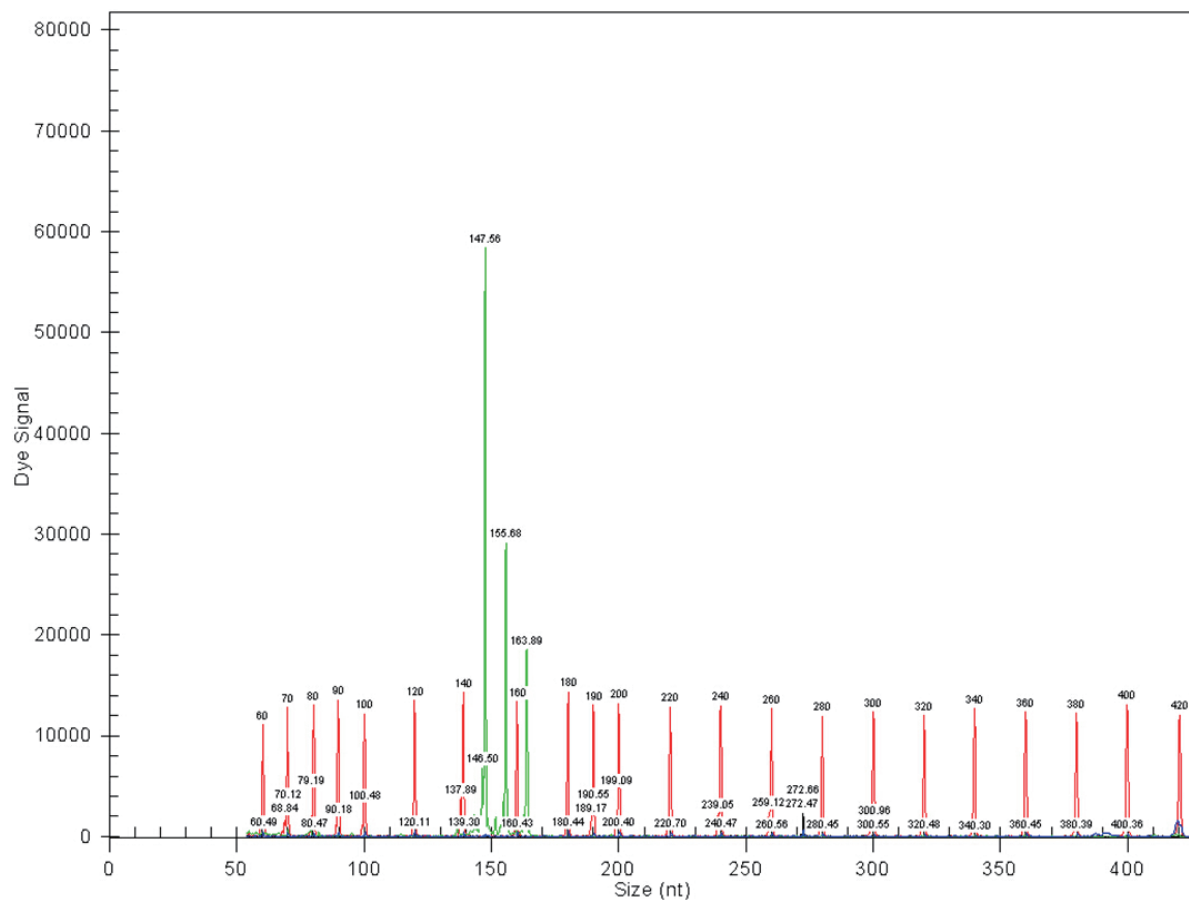
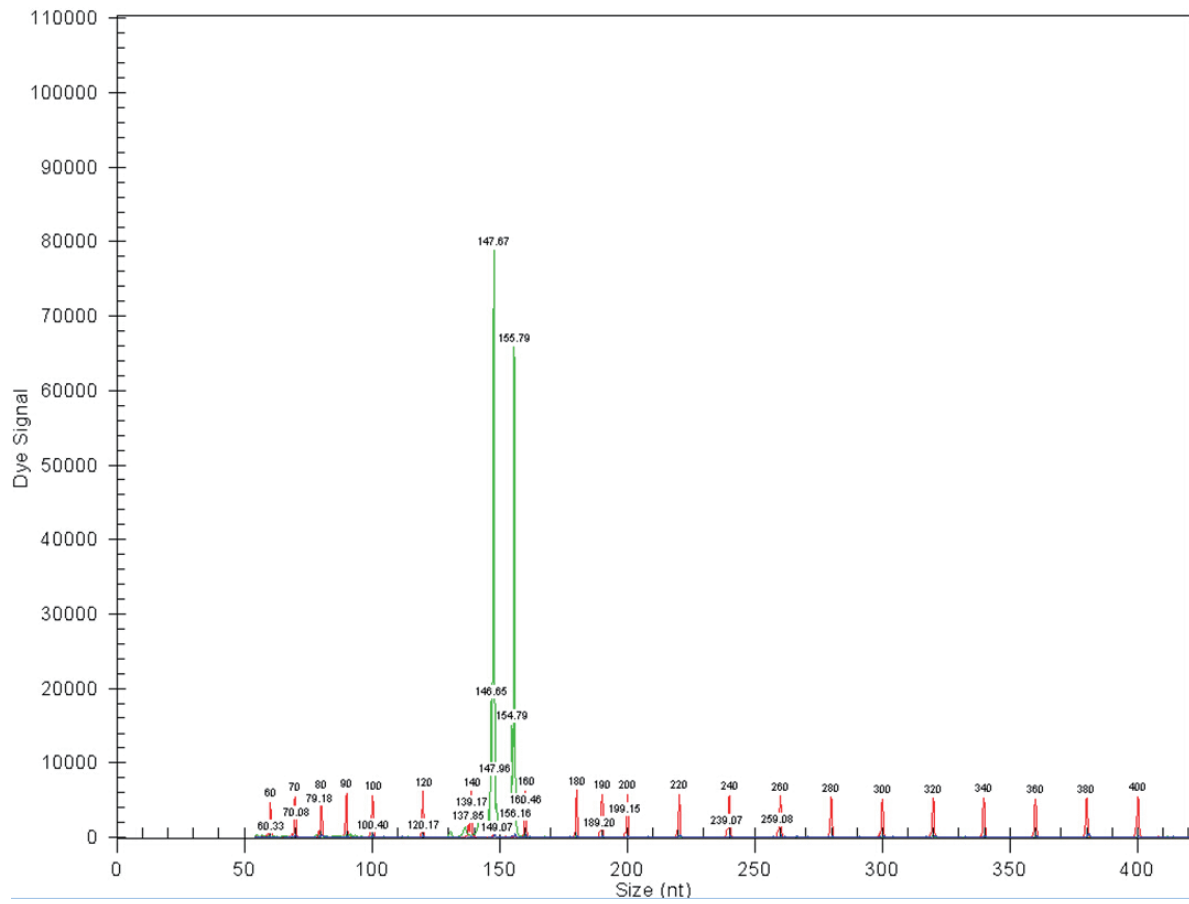


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**Figure S1** Results of genotyping of the marker D13S317 A) in the sample of the recipient TH-41 and B) in the follow-up biopsy. Results of genotyping of the marker D16S539 C) in the sample of the recipient TH-29 and D) in the follow-up biopsy. The sizes (183bp and 191bp) of the 2 peaks of the Figure S1A) are different from the sizes (174bp and 187bp) of the 2 peaks in Figure S1B). These results discard chimerism.

## DISCUSSION

According to the present study, the *IL28B* genotype of both, the recipient and the donor, influences response to the post-transplant treatment in patients infected with HCV. This conclusion is based in the similar rate of response that is found among the pairs CC/CC CC/non-CC and non-CC/CC. Only the combination non-CC/non-CC had a significantly worse rate of response. Our results support this conclusion in response to short and long term (ETR and SVR). In fact, although the IFN- $\lambda$  receptor is expressed by a limited number of cell types, any cell infected with HCV can produce this molecule<sup>[20]</sup>. Therefore, the effect of this polymorphism, may influence the response even in single dose and independently of the origin (recipient/graft).

Although many studies have provided evidence of the role of *IL28B* variants in the outcome of chronic HCV infection, there is much less information regarding the influence of these variants in the post liver transplantation treatment and, in addition, different studies had conflicting results<sup>[9,10,18,19]</sup>. In fact, according with some studies, the combinations recipient/donor with one risk factor (CC/ non-CC or non-CC/ CC) have the same rate of SVR than the combinations with two (non-CC/non-CC)<sup>[8,21]</sup>, whereas in others studies, the group with one factor has an intermediate rate of response between the other two groups<sup>[9,10,22]</sup>. In the present study, combinations recipient/donor with one risk factor have the same rate of response than those combinations having zero factors (CC/CC). Moreover, considering only the group with one risk factor, there are also conflicting results. Thereby, some studies show differences depending on the origin of the risk factor (recipient or donor), in some cases with worse response when the risk factor is from the recipient<sup>[21,22]</sup> whereas in others the worse response is related with the presence of the risk factor in the donor<sup>[10]</sup>. In our study and others, no differences related to the origin of the risk factor were observed<sup>[8,9]</sup>. These discrepancies may be due to bias introduced by the chimerism, ie. erroneous results in the assign of the genotype of the donor when biopsy is used as source (because of mix with genetic material of the recipient). This problem was detected in 6 of our pairs, which is almost 10% of the cases, which were removed of our study. In all the previous studies, follow-up biopsies were used to assign the genotype of the donor at least in some cases<sup>[8-10,21,22]</sup> but without performing any procedure to avoid the chimerism problem. So, this problem could explain, at least in part, the differences in the results among studies, taking into account that the number of pairs included in each individual work is relatively low. In this sense, it would be especially important that the papers show the results of the frequency of the *IL28B* genotypes in recipients and donors in order to demonstrate that they match with those expected. Thereby, a different distribution of *IL28B* genotypes in patients and donors, as occurs in our cohort, supports the reability of data, since the donor distribution reflects the frequencies in the general population, while patients requiring transplants are those having a worse response, therefore, this group has a lower frequency of individuals with a better response (CC).

Although *IL28B* genotyping has lost practical utility with the new highly efficacious regimens of treatment IFN-free, this kind of studies could supply basic information about the influence that the recipient and the graft have in the outcome of liver transplantation as well as in the response to treatment. Moreover, treatment response in transplanted patients is poorer results than in non-transplanted. Therefore, it is necessary to investigate the factors making these different results.

In summary, our results suggest that the response to the antiviral therapy post liver transplantation is associated to the *IL28B* genotype of both, the recipient and the donor.

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**Authorship:** MFTN: Conducted the study, analyzed the data and wrote the manuscript; MAMC: Designed, conducted the study, analyzed the data and wrote the manuscript; JMPA: conducted the study, analyzed the data and wrote the manuscript; JMSM: conducted the study; FGC: conducted the study; JRGL: Conducted the study, analyzed the data; MAGB: conducted the study; MFGE: Designed, conducted the study, analyzed the data and wrote the manuscript.

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## CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

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