Differential interferon signaling in cells in liver lobules and portal areas under treatment for chronic hepatitis C

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Running title: Gene expression under treatment for HCV

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Abbreviations: HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; miRNA, micro RNA; CH-B, Chronic Hepatitis B; CH-C, Chronic Hepatitis C; HCC-B, Hepatitis B-related Hepatocellular Carcinoma; HCC-C, Hepatitis C-related Hepatocellular Carcinoma; OCT, optimum cutting temperature

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Abstract

Objective: The mechanisms of treatment resistance to interferon (IFN) and ribavirin (Rib) combination therapy for hepatitis C virus (HCV) infection are not known. In this study, we therefore explored hepatic gene expression before and during treatment. ***Methods:*** Liver biopsy was performed in 50 patients before therapy and repeated in 30 patients one week after initiating combination therapy. The cells in liver lobules (CLL) and the cells in portal areas (CPA) were obtained from 12 patients using laser capture microdissection (LCM). ***Results:*** Forty-three patients were infected with genotype 1 HCV, 20 of whom were viral responders (genotype 1-Rps) with treatment outcome of SVR or TR, while 23 were non-viral responders (genotype 1-nonRsp) with NR. Only seven patients were infected with genotype 2. Before treatment, the expression of *IFN and Rib stimulated genes* (IRSGs), apoptosis-associated genes, and immune reaction gene pathways were greater in genotype 1-nonRsp than Rsp. During treatment, IRSGs were induced in genotype 1-Rsp, but not in nonRsp. IRSGs induction was irrelevant in genotype 2-Rsp and was mainly impaired in CLL but not in CPA. Pathway analysis revealed that many immune regulatory pathways were induced in CLL from genotype 1-Rsp, while growth factors related to angiogenesis and fibrogenesis were more induced in CPA from genotype 1-nonRsp. ***Conclusions****:* Impaired IRSGs induction in CLL reduces the sensitivity to treatment for genotype 1 HCV infection. CLL and CPA in the liver might be differentially involved in treatment resistance. These findings could be useful for the improvement of therapy for HCV infection.

Introduction

A human liver infected with hepatitis C virus (HCV) develops chronic hepatitis, cirrhosis, and in some instances, hepatocellular carcinoma (HCC). Although interferon (IFN) and ribavirin (Rib) combination therapy has become a popular modality for treating patients with chronic hepatitis C (CH-C), about 50% of patients relapse, particularly those with genotype 1b and high viral load [8]. The reasons for treatment failure are poorly understood. Many studies of IFN and Rib combination therapy for CH-C suggested that patients who cleared HCV viremia early during therapy tended to show favorable outcomes. On the other hand, patients who needed a longer period to clear HCV had poorer outcomes [4] [7] [17], and those who showed no response (no or minimal decrease in HCV-RNA) to IFN and Rib combination therapy hardly ever achieved a sustained viral response (SVR).

To elucidate the underlying mechanism of treatment resistance, expression profiles in the liver [3] [6] [20] and peripheral mononuclear cells (PBMC) [10] [21] during IFN treatment for CH-C patients have been examined. In chronic viral hepatitis, increased numbers of immune regulatory cells infiltrate in liver and liver-infiltrating lymphocytes (LILs) might play important roles for virus eradication and are potentially linked to treatment outcome. Previously, we selectively isolated cells in liver lobules (CLL) and cells in the portal area (CPA) from biopsy specimens using laser capture microdissection (LCM) and analyzed their gene expression profiles [11, 19]. From these analyses, it could be speculated that the majority of CLL was hepatocytes and the majority of CPA was lymphocytes, although other cellular components such as Kupffer cells, endothelial cells, myofibroblasts, and bile duct cells co-existed as well.

In this study, to investigate further insights into the mechanisms of therapy resistance, we analyzed expression profiles in CLL and CPA in addition to whole liver tissues during IFN therapy for CH-C.

Material and Methods

Patients

Patients with CH-C were enrolled in this study at the Graduate School of Medicine, Kanazawa University Hospital, Japan, between 2001 and 2007 (Table 1, Table 2). Informed consent was obtained from all patients and ethics approval for the study was obtained from the ethics committee for human genome/gene analysis research at Kanazawa University Graduate School of Medical Science. Thirty patients were administrated IFN-α 2b (6 MU: every day for 2 weeks, then three times a week for 22 weeks) (Schering-Plough K.K., Tokyo, Japan) and Rib (10-13 mg/kg/day) combination therapy for 24 weeks (Table 1). Twenty patients were administered Peg-IFN-α 2b and Rib combination therapy for 48 weeks (Table 2). The final outcome of the treatment was assessed at 24 weeks after cessation of combination therapy. In addition, ten normal liver tissues samples obtained during surgery for metastatic liver cancer were used as control samples.

We defined treatment outcomes according to the decrease in viremia as follows: sustained viral response (SVR), clearance of HCV viremia at 24 weeks after cessation of therapy; transient response (TR), no detectable HCV viremia at 24 weeks but relapse during the follow-up period; and nonresponse (NR), HCV viremia detected at the cessation of therapy. We defined a patient who achieved SVR or TR as a viral responder (Rsp) and a patient who exhibited a NR as a non-viral responder (nonRsp). As patient 10 stopped treatment at 5 weeks due to an adverse side effect, we grouped this patient as Rsp based on the observed viral decline within 2 weeks (Table 1).

HCV genotype was classified by the methods described by Okamoto et al. [16] Twenty-three patients were infected with genotype 1b and seven patients were infected with genotype 2 (2a; 6, 2b; 1) (Table 1, Table 2).

Patient serum was aliquoted and stored at -20˚C until use. HCV-RNA was serially monitored by quantitative real time detection (RTD)-PCR (COBAS® AmpliPrep/COBAS®

TaqMan® System®) [9] before treatment, at 48 h, 2 weeks, and 24 weeks after initiation of therapy and at 24 weeks after cessation of therapy.

The grading and staging of chronic hepatitis were histologically assessed according to the method described by Desmet et al. (Table 1) [5].

Preparation of liver tissue samples

Liver biopsy samples were taken from all patients at around 1 week before treatment and at 1 week after starting therapy (Figure 1A). The biopsy samples were divided into three parts: the first part was immersed in formalin for histological assessment, the second was immediately frozen in liquid nitrogen tank for future RNA isolation, and the final part was frozen in OCT compound for LCM analysis and stored at –80°C until use. As a control, a liver tissue sample was surgically obtained from a patient who showed no clinical signs of hepatitis and was analyzed as described previously [11].

CLL and CPA were isolated by LCM using a CRI-337 (Cell Robotics, Albuquerque, NM, USA) (supplement Figure 1) from the liver biopsy specimens frozen in OCT compound. The detailed procedure for LCM is described in the supplemental material and methods and was performed as previously described [11] [19].

RNA isolation and Affymetrix gene chip analysis

Total RNA in each liver biopsy specimen was isolated using the RNAqueous® kit (Ambion, Austin, TX, USA). Total RNA in the specimens frozen for LCM was isolated with a carrier nucleic acid (20 ng poly C) using RNAqueous®-Micro (Ambion). The quality of the isolated RNA was estimated after electrophoresis using an Agilent 2001 bioanalyzer (Palo Alto, CA, USA). Aliquots of total RNA (50 ng) isolated from the liver biopsy specimens were subjected to amplification with the WT-Ovation™ Pico RNA Amplification System (NuGen, San Carlos, CA, USA) as recommended by the manufacturer. About 10 μg of cDNA was amplified from 50 ng total RNA, and 5 μg of cDNA was used for fragmentation and biotin labeling using the FL-Ovation™ cDNA Biotin Module V2 (NuGen) as recommended by the

manufacturer. The biotin-labeled cDNA was suspended in 220 μl of hybridization cocktail (NuGen), and 200 μl was used for the hybridization. Half of the total RNA isolated from the LCM specimens was amplified twice with the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0 (EPICENTRE, Madison, WI, USA). Twenty-five micrograms of amplified antisense RNA was used for biotin labeling according to the manufacturer's protocol *Biotin-X-X-NHS* (provided by EPICENTRE). The biotin-labeled aRNA was suspended in 300 μl of hybridization cocktail (Affymetrix Inc., Santa Clara, CA, USA), and 200 μl was used for the hybridization with the Affymetrix Human 133 Plus 2.0 microarray chip containing 54,675 probes. After stringent washing, the microarray chips were stained with streptavidin-phycoerythrin, and probe hybridization was determined using a GeneChip® Scanner 3000 (Affymetrix). Data files (CEL) were obtained with the GeneChip® Operating Software 1.4 (GCOS) (Affymetrix). All expression data was deposited in Gene Expression Omunibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) (NCBI) and the accession ID is GSM 425995. The experimental procedure is described in detail in the supplemental material and methods.

Statistical and pathway analysis of gene chip data

Statistical analysis and hierarchical clustering were performed by BRB-ArrayTools (http://linus.nci.nih.gov/BRB-ArrayTools.htm). A class comparison tool based on univariate or paired *t*-tests was used to find differentially expressed genes (P<0.005). To confirm statistical significance, 2000 random permutations were performed, and all of the *t*-tests were re-computed for each gene. The gene set comparison was analyzed using the BioCarta and KEGG pathways data bases. The Fisher and Kolmogorov-Smirnov tests were performed for statistical evaluation (P<0.005) (BRB-ArrayTools). Functional ontology enrichment analysis was performed to compare the Gene Ontology (GO) process distribution of differentially expressed genes (P<0.05) using MetaCore™ (GeneGo, St. Joseph, MI, USA).

For the comparison of standardized expression values among different pathway

groups, standard units (Z-score) of each gene expression value were calculated as:

$$Z_i = \frac{X_i - X_m}{S}$$

where X_i is the raw expression value, X_m is the mean of the expression values in the pathway, and S is the standard deviation of the expression values.

The standard units in each pathway were expressed as mean ± SEM. A *P*-value of less than 0.05 was considered significant. Multivariate analysis was performed using a logistic regression model with a stepwise method using JMP7 for Windows (SAS Institute, Cary, NC, USA).

Quantitative Real-time detection (RTD)-PCR

We performed quantitative real-time detection PCR (RTD)-PCR using TaqMan Universal Master Mix (PE Applied Biosystems, CA). Primer pairs and probes for Mx1, IFI44 and IFITM1, and GAPDH were obtained from TaqMan assay reagents library (Applied Biosystems, CA).

Results

Serial changes in HCV-RNA after initiation of therapy of IFN-α 2b and Rib combination therapy

Serial changes in HCV-RNA were monitored at 48 h, 2 weeks, and 24 weeks after the initiation of therapy (Table 1). The biphasic viral decline after initiation of IFN therapy has been characterized [14] [15] [18]. We calculated the first phase decline by comparing viral load before therapy and after 48 h, and the second phase decline by comparing viral load after 48 h and 2 weeks (Table 1) [14][15] [18]. Both the first and second phase declines could be associated with treatment outcome and interestingly, viral responders (Rsp) who achieved SVR or TR showed more than a 1-log drop of first phase decline (Log/24 h) and more than a

0.3-log drop of second phase decline (Log/w) (Table 1). In contrast, non-viral responders (nonRsp) who exhibited NR, failed to meet the criteria. The first phase decline of Rsp and nonRsp were 1.38±0.65 Log/24 h and 0.77±0.44 Log/24 h (p=0.005). The second phase decline of Rsp and nonRsp were 0.71±0.34 Log/w and 0.11±0.34 Log/w (p=0.0001) respectively. Therefore, the classification of Rsp or nonRsp according to the treatment outcome might be feasible based on the viral kinetics responding to IFN. All but one patient infected with genotype 2 HCV eliminated virus within 2 weeks. There were no significant differences in the degree of histological activity or staging, nor in the sex, age, or alanine aminotransferase (ALT) level among these patients (Table 1). The amount of HCV-RNA was significantly lower in genotype 2 patients (4.06 ± 0.32 Log IU/ml) than in genotype 1 patients (5.70 ± 1.10 Log IU/mL) (Table 1).

Identification of IFN-α 2b plus Rib-induced genes in the livers of patients with chronic hepatitis C infection

To identify the genes induced in the liver by combination treatment with IFN-α 2b plus Rib, the gene expression profiles from samples taken around 1 week before and 1 week after initiation of therapy were compared. The pairwise *t*-test comparison showed that 798 genes were up-regulated and 220 genes were down-regulated significantly (P<0.005). The 100 most up-regulated genes according to the p-values were selected and are listed in supplement Table 1. Many of the interferon-stimulated genes (ISGs), such as Myxovirus (influenza virus) resistance 1 (MX), 2',5'-oligoadenylate synthetase (OAS), chemokine (C-C motif) ligand 8 (CCL8), and interferon alpha-inducible protein 27 (IFI 27), were significantly induced (supplement Table 1). We designated these genes as *IFN and Rib stimulated genes* (IRSGs) and analyzed them further.

Hepatic gene expression and responsiveness to IFN-α 2b and Rib combination therapy

To investigate the relationship between hepatic gene expression and responsiveness to treatment, we applied nonsupervised learning methods and then performed hierarchical

clustering analysis using all expressed genes (n=34988) from samples taken before and 1 week after initiation of therapy. The hierarchical clustering analysis of all patients did not form clear clusters, while hierarchical clustering within genotype 1 patients formed two clusters comprising Rsp and nonRsp (data not shown).

The fold changes in expression of the 100 most up-regulated IRSGs before and during therapy were calculated and subjected to hierarchical clustering. The hierarchical clustering using fold induction of IRSGs clearly differentiated Rsp, who exhibited higher IRSGs induction, from nonRsp, as shown in Figure 1A and supplement Table 1. Despite the rapid virus decline in genotype 2 patients, IRSGs induction was not so evident in these patients.

Unexpectedly, the hierarchical clustering of IRSGs expression in samples taken before treatment showed a different pattern of gene expression (Figure 2B). The expression of IRSGs before treatment was significantly higher in nonRsp than in Rsp. Upon treatment, the expression of IRSGs was more induced in Rsp than in nonRsp (Figure 1C).

The findings were confirmed in patients who were administered Peg-IFN-α 2b and Rib combination therapy (Table 2). IRSGs expression was induced in CH-C infected livers and substantially up-regulated in nonRsp compared with Rsp (supplemental Figure 1). Multivariate logistic analysis including age, sex, fibrosis stage, activity, HCV-RNA, genotype, treatment regime, ALT and expression pattern of IRSGs (up or down) of 50 patients before treatment showed that genotype 2 ($p<0.0001$, Odds=$4x10^7$) and down-regulated IRSGs ($p<0.0001$, Odds=71.2) are significant variables associated with SVR.

Gene expression analysis in cells in liver lobules (CLL) and portal area (CPA)

To explore these findings in more detail, we examined the gene expression profiles of CLL and CPA that had been isolated separately from whole liver biopsy specimens of twelve patients, using the LCM method before and during treatment (Figure 2A). The representative differentially expressed genes between CLL and CPA are shown in supplemental Table 2-1

and Table 2-2. In CLL, liver-specific proteins and enzymes, such cytochrome P450, apolipoprotein, and transferrin etc., were all expressed. In CPA, cytokines, chemokines and lymphocyte surface markers, such as chemokine (C-X-C motif) receptor 4, interleukin-7 receptor and CD83 antigen etc., were all expressed (supplemental Table 2-1, 2-2). The results confirmed our previous results that gene expression profiling obtained from the lobular area was mostly of hepatocyte origin and that from the portal area was mostly of liver infiltrating lymphocyte origin [11] [19].

IRSGs expression in CLL and CPA from genotype 1-Rsp and nonRsp is shown in Figure 2B. In genotype 1-Rsp, IRSGs expression was significantly induced in both CLL and CPA by the treatment (Figure 2B, 2C). On the other hand, in genotype 1-nonRsp and genotype 2, IRSGs induction was impaired especially in CLL, while it was nearly preserved in cells in CPA from three of five patients (Figure 2B, 2C). Thus, IRSGs induction in CLL should play an essential role in the eradication of the virus in genotype 1 CH-C patients.

Pathway analysis of gene expression in the livers of genotype 1-Rsp, genotype 1-nonRsp and genotype 2

To explore which signaling pathway contributed to the impaired IRSGs induction, pathway comparisons between genotype 1-Rsp (n=20) and genotype 1-nonRsp (n=23) before treatment were performed (Table 3). Gene set comparison was analyzed based on the database of BioCarta and KEGG pathways. The Fisher and Kolmogorov-Smirnov tests were performed for statistical evaluation (P<0.005) (BRB-ArrayTools). The mean probe intensities of representative genes in individual pathways are shown in Table 3. In genotype 1-nonRsp, the signaling pathways of IFN-α, apoptosis, and many of the immune pathways, such as those involved in antigen presentation, and the toll-like receptor (TRL) and Jak-STAT signaling pathways, were generally expressed at significantly higher levels before treatment than genotype 1-Rsp (Table 3, Figure 3). During treatment, the immune pathways were significantly up-regulated in genotype 1-Rsp, while in genotype 1-nonRsp and genotype 2

were not (Figure 3, whole liver). When the CLL and CPA were analyzed separately, significant induction of these pathways was observed in CLL of genotype 1-Rsp but not of genotype 1-nonRsp and genotype 2 (Figure 3, CLL). However, similar induction patterns were observed in CPA among genotype 1-Rsp, genotype1-nonRsp, and genotype 2 patients (Figure 3, CPA). Thus, these immune pathways should be activated in CLL for the elimination of virus.

We then evaluated the extent of the innate immune response to treatment. The expression of ten innate immune response genes was strongly induced in CLL from patients of genotype 1-Rsp but not from genotype 1-nonRsp and genotype 2 patients, although these genes were similarly induced in CPA among these patients (supplemental Table 3, Figure 3).

To examine which signaling pathways were differentially induced during treatment, we utilized MetaCore™. MetaCore™ is more feasible for pathway analysis using a relatively low number of cases, and was therefore selected to analyze the LCM samples in this study. The network processes involving genes for which the differential expression was statistically significant (P<0.05) in genotype 1 patients is shown in Figure 4. Before treatment, many of the immune mediated pathways, such as IFN-α, cell adhesion, IFN-γ, and TCR, were up-regulated in whole liver specimens from genotype 1-nonRsp compared with Rsp. Similar immune mediated pathways were up-regulated in CLL of genotype 1-nonRsp. In CPA, more of the pathways associated with cell proliferation and DNA damage were up-regulated, reflecting the active inflammatory process in the lymphocytes of genotype 1-nonRsp (Figure 4-A, B, C). During treatment, more of the immune reactive pathways, such as IFN, NK cell, and antigen presenting, were induced in the whole liver and CLL specimens from genotype 1-Rsp but not in nonRsp (Figure 4-D, E). In contrast, the expression of IFN-inhibitory genes was significantly induced in CLL from nonRsp during treatment (Table 3, Figure 4). Interestingly, in CPA, the IFN pathway was induced in genotype 1-Rsp and nonRsp to the same degree; however, signaling pathways related to angiogenesis and fibrogenesis, such as FGF, Wnt, TGF-beta, Nocth, and VEGF signaling, were induced more in CPA from genotype

1-nonRsp than from Rsp (Figure 4-F, Figure 3). Thus, differential expression of signaling pathways could be observed in CLL and CPA obtained from genotype 1-Rsp and nonRsp.

Discussion

IFN and Rib combination therapy has become a commonly used modality for treating patients with CH-C; however, the precise mechanism of treatment resistance has not yet been clarified. Since the development of quantitative assessment of viral kinetics during treatment, it has been demonstrated that patients who cleared HCV in the early period showed favorable outcomes, whereas patients who needed a longer time to clear HCV experienced poor outcomes [4] [7] [17]. Thus, early clearance of virus after initiation of treatment is one of the important determinants for complete eradication of HCV.

In this study, we analyzed gene expression from liver biopsy samples obtained before and at one week after initiation of treatment to investigate the precise mechanisms involved in treatment and treatment resistance. Although global gene expression profiles in the liver and PBMC during the IFN treatment in a chimpanzee has been reported [12][13], the relationship between the expression profiles and clinical outcome could not be evaluated.

During the preparation of this study, two reports using a similar approach have been published [6] [20]. For example, Feld et al. [6] analyzed gene expression in the livers of CH-C patients on treatment. However, the authors compared gene expression among different patients at initiation (n=19; 5 rapid responders, 10 slow responders, 4 naive) and during treatment (n=11; 6 rapid responders, 5 slow responders). Because patients were not serially biopsied before and during the treatment, true treatment-related gene induction could not be evaluated. Moreover, half of the on-treatment group was administered Rib alone for three days prior to liver biopsy. In the other report, Sarasin-Filipowicz et al. [20] extensively analyzed serial liver biopsy specimens under the treatment; however, the number of the patients enrolled in their study was relatively low and heterogeneous with respect to the

infected genotypes. Our study has extended their findings and provides further insights into the mechanism of IFN-resistance by analyzing gene expression in CLL and CPA separately for the first time. The analysis of genotype 2 HCV enabled us to understand the importance of the differing sensitivities to IFN between strains.

By comparing gene expression in serial liver biopsy specimens obtained at the initiation and during treatment, IFN- and Rib-stimulated genes (IRSGs) in the livers of patients with CH-C could be identified (supplement Table 1). Our study clearly demonstrated that IRSGs induction correlated with the elimination of HCV in patients with genotype 1 in accordance with previous results [6] [20]. The patients who did not show a response to treatment had poor induction of IRSGs (Figure 1A). In contrast, IRSGs expression before treatment showed an opposite pattern of expression. IRSGs were induced in genotype 1-nonRsp rather than in genotype 1-Rsp. This finding was first described by Chen et al. [3] and confirmed by others [1] [6] [20]. Asselah et al. [1] extensively analyzed 58 curated ISGs published previously by RTD-PCR and found that three genes (IFI27, CXCL9 and IFI-6-16) were predictive of treatment outcome. However, only 12 of their 58 curated genes were also included in the 100 most up-regulated genes we observed during treatment (supplement Table 1). Therefore, more valuable genes for the prediction of treatment outcome might exist and our gene list could be useful for further selection of predictors of treatment outcome.

We showed that different levels of IRSGs induction before treatment was associated with up-regulation of different signaling pathways, such as apoptosis and inflammatory pathways, in genotype 1-nonRsp, although histological assessment of activities and stages could not differentiate the two groups of patients. During treatment, these pathways, including the innate immune response for IFN production, were significantly induced in genotype 1-Rsp but not in genotype 1-nonRsp. The results suggest that previous up-regulation of IRSGs might be linked to impaired induction of IRSGs and contribute to the poor response to treatment in patients with genotype 1. Interestingly, an impaired IRSGs induction was mainly noticeable in

CLL, but not in CPA, and the results were confirmed by RTD-PCR (data not shown). These results suggest that IRSGs induction in HCV-infected hepatocytes could play an essential role in the eradication of the genotype 1 virus in CH-C patients.

However, these scenarios did not apply in patients with genotype 2 HCV in this study. Despite the presence of active inflammation before treatment and unsatisfactory IRSGs induction during treatment, these patients showed rapid responses to treatment and favorable treatment outcomes. It could be speculated that genotype 2 HCV is far more sensitive to IFN than genotype 1 HCV, and small IRSGs induction might be enough to eradicate virus. Further studies are needed to confirm these results.

We precisely analyzed the expression profiles in CLL and CPA obtained using the LCM method. Although IRSGs and other immune regulatory genes were similarly induced in the CPA of genotype 1-Rsp and nonRsp, more of the angiogenic- and fibrogenic-related genes were induced in CPA of genotype 1-nonRsp (Figure 4C, F). Therefore, growth factors released from CPA might be involved in poor IRSGs induction in CLL of genotype 1-nonRsp.

In summary, by comparing the hepatic gene expression in CH-C patients with different treatment outcomes, we identified a gene expression signature characteristic of IFN resistance. Our study is very important for two reasons: it will help in the development of new therapeutic strategies and many of the genes found to be up-regulated between genotype 1-Rsp and nonRsp encode molecules secreted in serum (cytokines). Therefore, the study represents a logical functional approach for the development of serum markers as predictors of response to treatment [2]. The precise mechanisms underlying these findings should be clarified further in future studies.

References

[1] Asselah T, Bieche I, Narguet S, Sabbagh A, Laurendeau I, Ripault MP, et al. Liver gene expression signature to predict response to pegylated interferon plus ribavirin combination therapy in patients with chronic hepatitis C. Gut 2008;57(4): 516-524.

[2] Asselah T, Bieche I, Sabbagh A, Bedossa P, Moreau R, Valla D, et al. Gene expression and hepatitis C virus infection. Gut 2009;58(6): 846-858.

[3] Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology 2005;128(5): 1437-1444.

[4] Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38(3): 645-652.

[5] Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19: 1513-1520.

[6] Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. Hepatology 2007;46(5): 1548-1563.

[7] Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Goncales FL, Jr., et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. J Hepatol 2005;43(3): 425-433.

[8] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13): 975-982.

[9] Germer JJ, Harmsen WS, Mandrekar JN, Mitchell PS, Yao JD. Evaluation of the

COBAS TaqMan HCV test with automated sample processing using the MagNA pure LC instrument. J Clin Microbiol 2005;43(1): 293-298.

[10] He XS, Ji X, Hale MB, Cheung R, Ahmed A, Guo Y, et al. Global transcriptional response to interferon is a determinant of HCV treatment outcome and is modified by race. Hepatology 2006;44(2): 352-359.

[11] Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Kaneko S. Different signaling pathways in the livers of patients with chronic hepatitis B or chronic hepatitis C. Hepatology 2006;44(5): 1122-1138.

[12] Huang Y, Feld JJ, Sapp RK, Nanda S, Lin JH, Blatt LM, et al. Defective hepatic response to interferon and activation of suppressor of cytokine signaling 3 in chronic hepatitis C. Gastroenterology 2007;132(2): 733-744.

[13] Lanford RE, Guerra B, Bigger CB, Lee H, Chavez D, Brasky KM. Lack of response to exogenous interferon-alpha in the liver of chimpanzees chronically infected with hepatitis C virus. Hepatology 2007;46(4): 999-1008.

[14] Layden TJ, Layden JE, Reddy KR, Levy-Drummer RS, Poulakos J, Neumann AU. Induction therapy with consensus interferon (CIFN) does not improve sustained virologic response in chronic hepatitis C. J Viral Hepat 2002;9(5): 334-339.

[15] Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 1998;282(5386): 103-107.

[16] Okamoto H, Tokita H, Sakamoto M, Horikita M, Kojima M, Iizuka H, et al. Characterization of the genomic sequence of type V (or 3a) hepatitis C virus isolates and PCR primers for specific detection. J Gen Virol 1993;74 (Pt 11): 2385-2390.

[17] Payan C, Pivert A, Morand P, Fafi-Kremer S, Carrat F, Pol S, et al. Rapid and early virological response to chronic hepatitis C treatment with IFN alpha2b or PEG-IFN alpha2b plus ribavirin in HIV/HCV co-infected patients. Gut 2007;56(8): 1111-1116.

[18] Rosen HR, Ribeiro RR, Weinberger L, Wolf S, Chung M, Gretch DR, et al. Early hepatitis C viral kinetics correlate with long-term outcome in patients receiving high dose induction followed by combination interferon and ribavirin therapy. J Hepatol 2002;37(1): 124-130.

[19] Sakai Y, Honda M, Fujinaga H, Tatsumi I, Mizukoshi E, Nakamoto Y, et al. Common transcriptional signature of tumor-infiltrating mononuclear inflammatory cells and peripheral blood mononuclear cells in hepatocellular carcinoma patients. Cancer Res 2008;68(24): 10267-10279.

[20] Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, et al. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci U S A 2008;105(19): 7034-7039.

[21] Younossi ZM, Baranova A, Afendy A, Collantes R, Stepanova M, Manyam G, et al. Early gene expression profiles of patients with chronic hepatitis C treated with pegylated interferon-alfa and ribavirin. Hepatology 2009;49(3): 763-774.

Legends to figures

Figure 1

A: Hierarchical clustering of expression in genotype 1 and genotype 2 patients during treatment according to fold induction of IRSGs.

B: Hierarchical clustering of expression in genotype 1 and genotype 2 patients before treatment.

C: Serial changes in standardized expression values (Z-score) of IRSGs from genotype 1-Rsp, genotype 1-nonRsp, and genotype 2 patients before and during treatment.

Figure 2

A: LCM of liver biopsy samples before and during treatment.

B: Heat map of gene expression of IRSGs in CLL and CPA before and during treatment.

C: Serial changes in standardized expression values (Z-score) of IRSGs in CLL and CPA from genotype 1-Rsp, genotype 1-nonRsp, and genotype 2 patients before and during treatment.

Figure 3

Serial changes in standardized expression values (Z-score) of differentially expressed pathways from genotype 1-Rsp, genotype 1-nonRsp, and genotype 2 patients before and during treatment in whole liver, CLL, and CPA.

Figure 4

Functional ontology enrichment analysis of differentially expressed genes ($p<0.05$) using MetaCore™. GeneGo network process of differentially expressed genes between genotype 1-Rsp (orange bar) and genotype 1-nonRsp (blue bar) are listed in order of decreasing statistical significance.

Supplementary Figure 1

IRSGs expression in the liver of control patients, genotype 1-Rsp and nonRsp who received Peg-IFN-α 2b and Rib therapy.

Table1

Table 1. Characteristics of study patients who received IFN and ribavirin combination therapy

Pt.No.	Sex	Age (yr)	Genotype	ALT (IU/mL)		Liver histology				LCM	HCV-RNA (Log IU/mL)				Viral kinetics		Viral response	Outcome
				Before therapy	During therapy	Before therapy		During therapy			Before therapy	48 h	2 wk	24 wk	1st phase delinie	2nd phase decline		
						F	A	F	A						Log/24 h	Log/week		
1	M	48	1b	83	45	1	1	1	1	+	6.6	4.5	3.5	-	1.1	0.5	Rsp	SVR
2	M	32	1b	192	95	1	1	1	1	-	6.4	3.9	3.2	-	1.3	0.4	Rsp	SVR
3	F	50	1b	57	37	1	1	1	1	-	5.8	2.5	1.5	-	1.7	0.5	Rsp	TR
4	M	36	1b	119	117	1	1	1	1	+	6.1	4.4	4.2	+	0.9	0.1	nonRsp	NR
5	M	54	1b	82	69	1	1	1	1	-	6.6	5.1	3.9	+	0.8	0.6	nonRsp	NR
6	M	43	1b	143	116	1	1	1	1	-	6.3	4.4	4.1	+	1.0	0.2	nonRsp	NR
7	M	48	1b	33	30	1	1	1	1	+	1.5	0.0	0.0	-	>0.8	-	Rsp	SVR
8	M	52	1b	316	374	1	2	1	1	-	4.7	5.1	3.9	+	-0.2	0.6	nonRsp	NR
9	M	45	1b	112	39	1	0	2	0	-	6.2	5.1	5.7	+	0.6	-0.3	nonRsp	NR
10	M	48	1b	48	30	2	2	2	1	+	6.4	4.0	2.6	NA	1.2	0.8	Rsp	NA
11	M	52	1b	114	80	2	2	2	1	-	6.1	3.7	3.0	-	1.2	0.4	Rsp	TR
12	F	63	1b	38	30	2	1	2	1	-	5.2	4.2	4.5	+	0.5	-0.2	nonRsp	NR
13	M	58	1b	90	83	2	2	2	2	+	6.9	4.9	5.6	+	1.0	-0.4	nonRsp	NR
14	F	61	1b	87	43	2	1	2	1	+	6.5	3.9	3.7	+	1.3	0.1	nonRsp	NR
15	F	64	1b	133	111	2	1	3	2	-	6.0	4.4	3.6	+	0.8	0.4	nonRsp	NR
16	F	62	1b	251	159	3	2	3	2	-	4.8	2.7	1.5	-	1.1	0.6	Rsp	SVR
17	M	54	1b	211	205	3	2	3	2	+	6.7	0.0	0.0	-	>3.4	-	Rsp	SVR
18	F	68	1b	153	145	3	2	3	2	+	4.9	4.3	3.5	+	0.3	0.4	nonRsp	NR
19	F	69	1b	64	43	3	2	3	2	-	4.4	1.5	0.0	-	1.5	0.8	Rsp	SVR
20	M	49	1b	91	83	3	2	3	2	+	6.6	4.2	3.8	+	1.2	0.2	nonRsp	NR
21	M	55	1b	187	196	4	1	4	2	-	5.8	5.1	5.6	+	0.4	-0.3	nonRsp	NR
22	F	45	1b	113	75	4	2	3	3	-	5.7	4.2	2.7	-	0.8	0.8	Rsp	TR
23	M	60	1b	86	49	4	2	3	1	-	6.3	3.5	3.5	+	1.4	0.0	nonRsp	NR
24	F	51	2b	98	90	1	1	1	1	-	2.7	1.5	0.0	-	0.6	0.8	Rsp	SVR
25	M	37	2a	241	211	1	0	1	0	-	4.0	1.5	0.0	-	1.3	0.8	Rsp	SVR
26	F	45	2a	91	33	2	1	2	1	-	5.4	2.2	1.5	-	1.6	0.4	Rsp	TR
27	M	46	2a	101	45	2	1	2	1	+	3.6	0.0	0.0	-	>1.8	-	Rsp	SVR
28	M	54	2a	196	177	3	2	2	1	+	4.2	0.0	0.0	-	>2.1	-	Rsp	SVR
29	F	68	2a	234	135	3	1	3	2	+	4.6	3.1	0.0	-	0.8	1.7	Rsp	SVR
30	M	67	2a	155	163	4	2	4	2	-	3.9	1.5	0.0	-	1.2	0.8	Rsp	SVR

NA : not applicable
LCM: laser capture microdissection
ALT: alanine aminotransferase
A: activity
F: fibrosis

1st phase decline was determined by subtracting HCV-RNA at 48 h from before therapy.
2nd phase decline was determined by subtracting HCV-RNA at 2 wk from 48 h.
SVR: sustained viral response
NR: nonresponse
TR: transient response
Rsp: viral responder, patients with SVR or TR
nonRsp: non-viral responder; patients with NR
HCV RNA was assayed by COBAS® AmpliPrep/COBAS® TaqMan® System® (Log IU/mL)

Table2

Table 2. Characteristics of patients who received Peg-IFN and ribavirin combination therapy and normal control

Pt.No.	Sex	Age (yr)	Genotype	ALT (IU/mL)	Liver histology		HCV-RNA (Log IU/mL)				Viral response	Outcome
				Before therapy	Before therapy		Before therapy	2 wk	4 wk	24 wk		
					F	A						
1	M	57	1b	68	1	1	6.5	-	-	-	Rsp	SVR
2	F	56	1b	31	1	1	6.5	4.4	-	-	Rsp	SVR
3	M	63	1b	50	1	1	6.1	-	-	-	Rsp	SVR
4	M	44	1b	45	1	1	6.5	3.7	-	-	Rsp	SVR
5	F	51	1b	27	2	1	6.5	4.1	-	-	Rsp	SVR
6	M	58	1b	72	2	1	6.2	-	-	-	Rsp	SVR
7	M	60	1b	71	2	2	6.2	3.9	-	-	Rsp	SVR
8	F	52	1b	58	2	2	6.5	4.1	-	-	Rsp	SVR
9	F	62	1b	60	3	2	5.9	3.8	-	-	Rsp	SVR
10	M	55	1b	106	3	2	6.4	-	-	-	Rsp	SVR
11	M	30	1b	31	1	1	6.4	6.1	5.9	+	nonRsp	NR
12	F	55	1b	23	1	2	6.5	6.1	5.9	+	nonRsp	NR
13	M	58	1b	129	1	2	6.3	6.0	5.8	+	nonRsp	NR
14	M	42	1b	326	2	1	6.6	6.2	5.8	+	nonRsp	NR
15	F	61	1b	77	2	1	6.1	5.9	5.7	+	nonRsp	NR
16	F	44	1b	31	2	2	5.5	5.3	4.7	+	nonRsp	NR
17	M	51	1b	38	2	2	6.5	6.2	5.9	+	nonRsp	NR
18	F	55	1b	97	2	2	6.7	6.3	6.1	+	nonRsp	NR
19	M	59	1b	31	3	2	6.7	5.9	5.7	+	nonRsp	NR
20	F	53	1b	71	3	2	5.9	5.8	5.8	+	nonRsp	NR
21	F	51	-	18	0	0	-	-	-	-	-	-
22	F	78	-	13	0	0	-	-	-	-	-	-
23	M	75	-	20	0	0	-	-	-	-	-	-
24	M	34	-	12	0	0	-	-	-	-	-	-
25	M	64	-	30	0	0	-	-	-	-	-	-
26	M	78	-	9	0	0	-	-	-	-	-	-
27	M	53	-	19	0	0	-	-	-	-	-	-
28	F	64	-	12	0	0	-	-	-	-	-	-
29	F	60	-	20	0	0	-	-	-	-	-	-
30	M	66	-	26	0	0	-	-	-	-	-	-

SVR: sustained viral response
NR: nonresponse
Rsp: viral responder, patients with SVR or TR
nonRsp: non-viral responder; patients with NR

Table3

Table 3. Up- and down-regulated pathways by gene set comparison between Rsp and nonRsp of genotype 1 patients before therapy (BRB-array tool)

Pathway	No. of genes	LS p-value	KS p-value	Representative Genes	Mean probe intensity of representative genes		
					Rsp (n=20)	nonRsp (n=23)	Normal (n=10)
Up-regulated in slow viral drop							
IFN alpha signaling pathway	21	0.00001	0.00300	STAT1	1608	3117	686
				IRF9	1249	1842	614
				IFNAR2	1892	1988	903
Apoptotic Signaling in Response to DNA Damage	55	0.00001	0.07974	CASP3	675	870	426
				CASP7	1165	1510	1264
				CASP9	355	403	264
				TP53	1465	1797	1028
Toll-like receptor signaling pathway	150	0.00006	0.06659	CXCL10	1922	3979	193
				CXCL11	176	321	51
				MYD88	1022	1372	723
				TIRAP	582	722	447
Wnt signal pathway	55	0.00009	0.16058	EIF2AK2	664	1190	484
				CCND1	2439	3558	1162
				APC	143	186	154
				PIK3R1	1570	1906	682
Antigen processing and presentation	139	0.00117	0.00091	TAP2	169	317	93
				HLA-A	11005	14726	6221
				HLA-B	13144	17942	6823
				HLA-C	1937	3993	783
Jak-STAT signaling pathway	220	0.00180	0.13154	STAT2	716	1065	274
				IL28RA	390	544	204
				IL10RB	398	506	338
Down-regulated in slow viral drop							
Metabolism of xenobiotics by cytochrome P450	98	0.00018	0.00082	CYP3A4	15219	10118	19256
				CYP2E1	29129	24549	30929
				AKR1C4	6126	4898	6671
Fatty acid metabolism	88	0.00480	0.05373	ACADL	826	687	785
				ALDH2	18325	16337	21844
				HSD17B4	9619	8807	10653
				ACAD11	6858	6238	8279
				ACOX1	6988	5862	8279

No. of genes: the number of genes comprising the pathway
Rsp: viral responder, patients with SVR or TR
nonRsp: non viral responder; patients with NR

Fig. 1

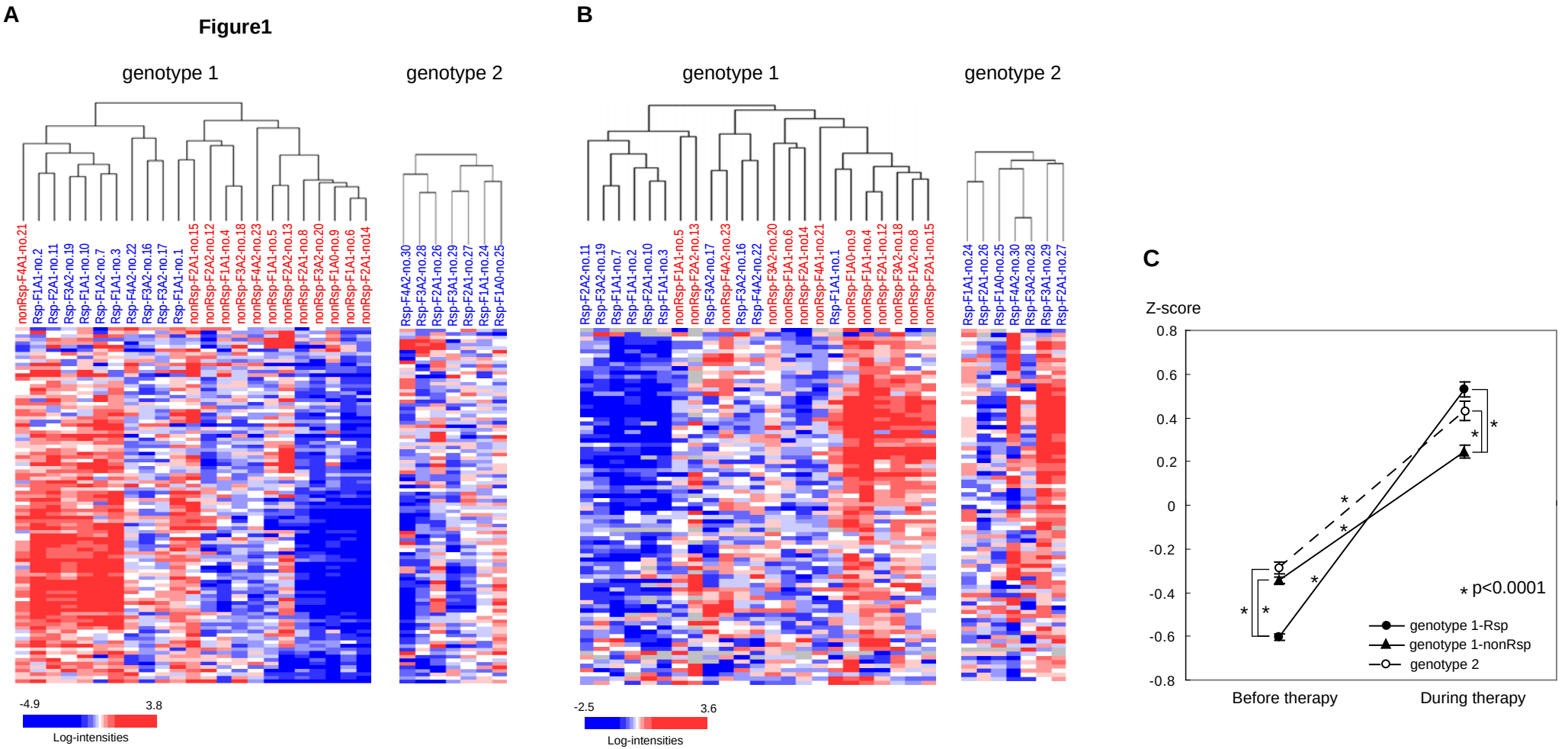

A
Figure1
genotype 1
genotype 2
nonRsp-F4A1-no.21
Rsp-F1A1-no.2
Rsp-F2A1-no.11
Rsp-F3A2-no.19
Rsp-F1A1-no.10
Rsp-F1A2-no.7
Rsp-F1A1-no.3
Rsp-F4A2-no.22
Rsp-F3A2-no.16
Rsp-F3A2-no.17
Rsp-F1A1-no.1
nonRsp-F2A1-no.15
nonRsp-F2A2-no.12
nonRsp-F1A1-no.4
nonRsp-F3A2-no.18
nonRsp-F4A2-no.23
nonRsp-F1A1-no.5
nonRsp-F2A2-no.13
nonRsp-F2A1-no.8
nonRsp-F3A2-no.20
nonRsp-F1A0-no.9
nonRsp-F1A1-no.6
nonRsp-F2A1-no14
Rsp-F4A2-no.30
Rsp-F3A2-no.28
Rsp-F2A1-no.26
Rsp-F3A1-no.29
Rsp-F2A1-no.27
Rsp-F1A1-no.24
Rsp-F1A0-no.25
-4.9
3.8
Log-intensities
B
genotype 1
genotype 2
Rsp-F2A2-no.11
Rsp-F3A2-no.19
Rsp-F1A1-no.7
Rsp-F1A1-no.2
Rsp-F2A1-no.10
Rsp-F1A1-no.3
nonRsp-F1A1-no.5
nonRsp-F2A2-no.13
Rsp-F3A2-no.17
nonRsp-F4A2-no.23
Rsp-F3A2-no.16
Rsp-F4A2-no.22
nonRsp-F3A2-no.20
nonRsp-F1A1-no.6
nonRsp-F2A1-no14
nonRsp-F4A1-no.21
Rsp-F1A1-no.1
nonRsp-F1A0-no.9
nonRsp-F1A1-no.4
nonRsp-F2A1-no.12
nonRsp-F3A2-no.18
nonRsp-F1A2-no.8
nonRsp-F2A1-no.15
Rsp-F1A1-no.24
Rsp-F2A1-no.26
Rsp-F1A0-no.25
Rsp-F4A2-no.30
Rsp-F3A2-no.28
Rsp-F3A1-no.29
Rsp-F2A1-no.27
-2.5
3.6
Log-intensities
C
Z-score
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
* p<0.0001
genotype 1-Rsp
genotype 1-nonRsp
genotype 2
Before therapy
During therapy

Figure2

Fig. 2

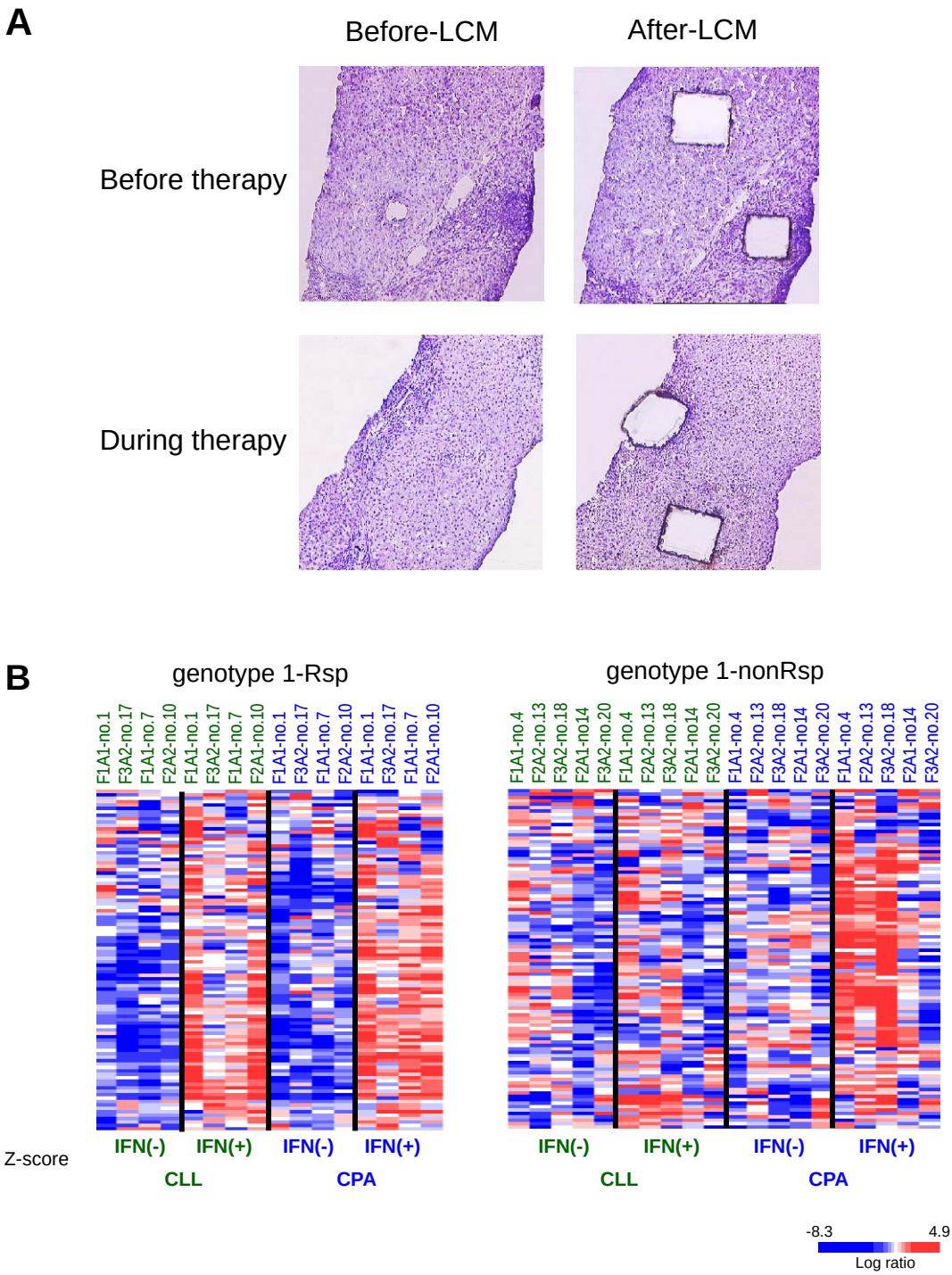


Fig. 3

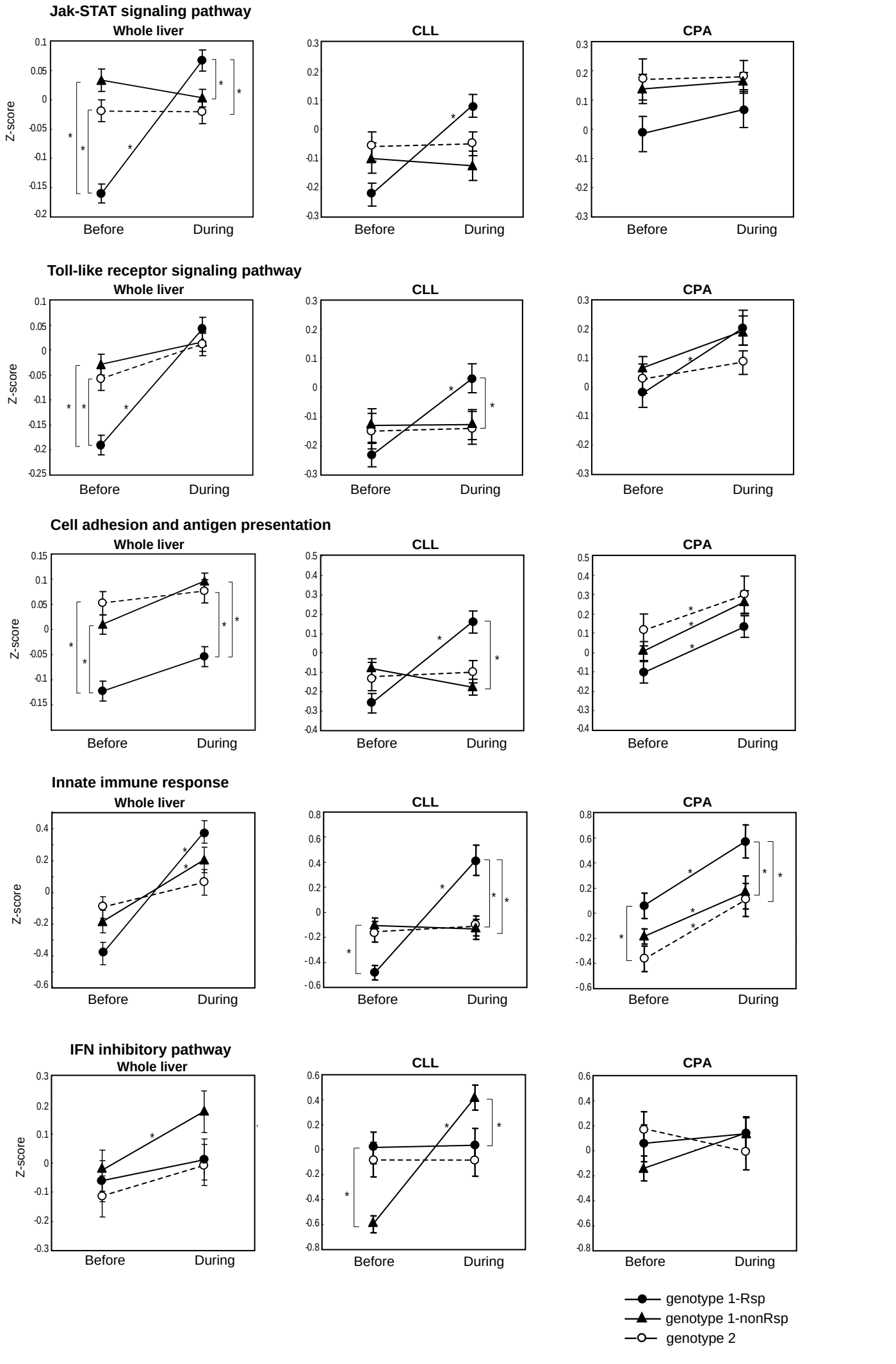
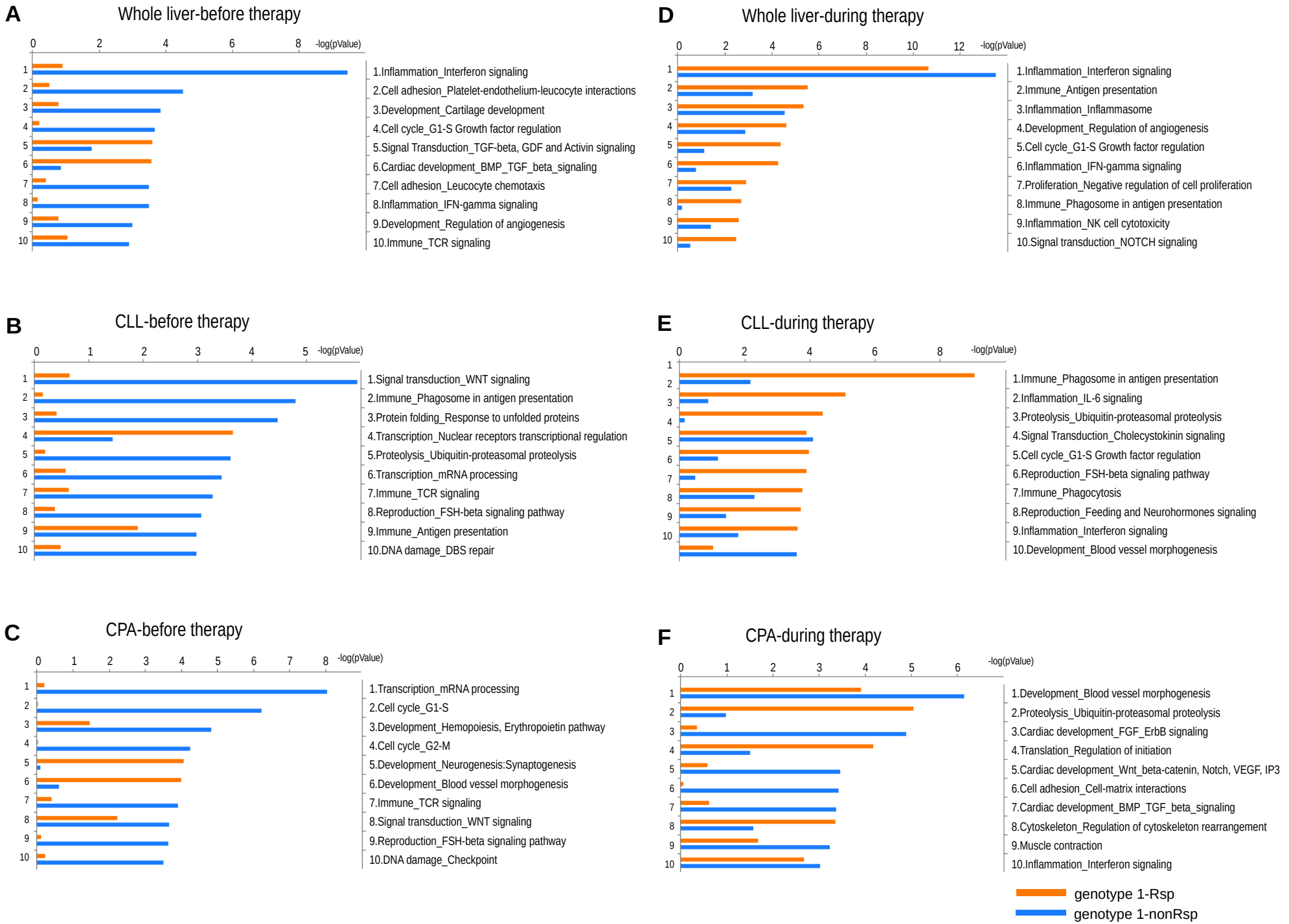

Jak-STAT signaling pathway
Whole liver
CLL
CPA
Toll-like receptor signaling pathway
Whole liver
CLL
CPA
Cell adhesion and antigen presentation
Whole liver
CLL
CPA
Innate immune response
Whole liver
CLL
CPA
IFN inhibitory pathway
Whole liver
CLL
CPA
Z-score
Before
During
genotype 1-Rsp
genotype 1-nonRsp
genotype 2

Figure4

Fig. 4



Supplemental material and methods

RNA isolation and Affymetrix gene chip analysis

The liver biopsy specimens stored in liquid nitrogen were once immersed in RNA*later*® (Ambion, Austin, TX, USA) over night and homogenized in lysis buffer by TissueLyser (Qiagen, Hiden, Germany). Total RNA was isolated using the RNAqueous® kit (Ambion, Austin, TX, USA). The quality of the isolated RNA was estimated after electrophoresis using an Agilent 2001 bioanalyzer (Palo Alto, CA, USA). Aliquots of total RNA (50 ng) isolated from the liver biopsy specimens were subjected to amplification with the WT-Ovation™ Pico RNA Amplification System (NuGen, San Carlos, CA, USA) as recommended by the manufacturer (http://www.nugeninc.com/). Briefly, double stranded cDNA was synthesized from template RNA by using unique SPIA™ DNA/RNA chimeric primer. The resultant cDNA with a unique DNA/RNA heterosuplex at one end was subjected to a linear isothermal DNA amplification under the presence of RNase H and DNA polymerase. RNase H degraded RNA in the DNA/RNA heterosuplex, generating single stranded DNA site at the 3' end for attaching a new chimeric primer and start synthesizing a single stranded cDNA. This amplification cycle continues to generate about 10 μg of cDNA from 50 ng total RNA. For the hybridization, 5 μg of resultant cDNA was used for fragmentation and biotin labeling using the FL-Ovation™ cDNA Biotin Module V2 (NuGen) as recommended by the manufacturer. The biotin-labeled cDNA was suspended in 220 μl of hybridization cocktail (NuGen), and 200μl was used for the hybridization. Affymetrix Human 133 Plus 2.0 microarray chip (Affymetrix, Santa Clara, CA, USA) containing 54,675 gene transcripts was used for the analysis. Hybridization (45°C for 16hrs at 60 rpm), washing and staining (using Fluidics Station) were performed according to the standard protocol (Affymetrix). The probe

array was scanned by GeneChip® Scanner 3000(Affymetrix). Hybridized data files (CEL) were obtained with the GeneChip® Operating Software 1.4 (GCOS) (Affymetrix).

For laser capture microdissection (LCM), the frozen liver biopsy specimens in O.C.T. compound were sliced into sections 8 μm thick and immediately fixed in methanol for 5 min and kept on dry ice as described previously.[10] Tissue samples were quickly stained with toluidine blue and dissected. Around 500 lymphoid cells and hepatocytes were excised on three slides and immersed in a denaturing solution. Dissection was completed within 5 min for each slide. Total RNA in LCM was isolated with a carrier nucleic acid (20ng poly C) using RNAqueous™-Micro (Ambion). The quality of the isolated RNA was estimated after electrophoresis using an Agilent 2001 bioanalyzer (Palo Alto, CA, USA). Usually, from 500 cells obtained, 20–40 ng total RNA was isolated. Half of the total RNA isolated from the LCM specimens was amplified twice with the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0 (*EPICENTRE*, Madison, WI, USA). The amplification protocol was based on the standard antisense RNA amplification procedure using T7 RNA polymerase and Eberwine oligo-dT/T7 primer. Two round amplification yielded 20–40 μg aRNA and 20 μg aRNA was used for further biotin labeling using *Biotin-X-X-NHS* (provided by *EPICENTRE*) according to the manufacturer's protocol. The biotin-labeled aRNA was suspended in 300 μl of hybridization cocktail (Affymetrix Inc, Santa Clara, CA, USA), and 200μl was used for the hybridization with the Affymetrix Human 133 Plus 2.0 microarray chip. Hybridization, washing, staining and scanning procedures were performed as described. Hybridized data files (CEL) were obtained with the GeneChip® Operating Software 1.4 (GCOS) (Affymetrix).

Processing of gene chip data

The intensity of each gene chip was normalized by one patient using GeneChip® Operating Software 1.4 (GCOS) and further processed by BRB-ArrayTools (http://linus.nci.nih.gov/BRB-ArrayTools.htm). The absent detection call was excluded and filtered data were log-transferred and normalized using median over the array (BRB-ArrayTools). Hierarchical clustering was performed using average linkage with centered correlation. A pared class comparison was performed to find the differentially expressed genes induced by the treatment. A gene set comparison comparison tool provides a list that has more genes differentially expressed and is coordinately regulated among predefined clinical groups than expected by chance and enables findings among biologically related genes to reinforce each other. The analysis was performed using the BioCarta and KEGG pathways data bases. The Fisher and Kolmogorov-Smirnov tests were performed for statistical evaluation (P<0.005) (BRB-ArrayTools).

In addition to BRB-ArrayTools, we performed the pathway analysis by using MetaCore™ (GeneGo, St. Joseph, MI, USA). Differentially expressed genes among predefined groups were extracted by using a class comparison tool (BRB-array tool) (P<0.05) and applied to MetaCore™ to find which category of genes were included. In MetaCore™, functional ontology enrichment was performed to compare the Gene Ontology (GO) process distribution of differentially expressed genes (P<0.05).

Supplementary Table 1. One hundred genes most up-regulated by IFN and Rib combination therapy in liver of patients with chronic hepatitis C

Probe Set	Symbol	Description	GO: Molecular Function	*p*-value	During/Before IFN			
					Total	genotype 1-Rsp	genotype 1-nonRsp	genotype2
214038_at	CCL8	Chemokine (C-C motif) ligand 8	inflammatory response	1.0E-08	10.0	10.0	5.0	5.0
204439_at	IFI44L	Interferon-induced protein 44-like	immune response	1.0E-08	5.0	10.0	2.5	3.3
229450_at	IFIT3	Interferon-induced protein with tetratricopeptide repeats 3	lipid metabolic process	1.0E-08	5.0	10.0	2.5	3.3
202086_at	MX1	Myxovirus (influenza virus) resistance 1	antiviral immune response	1.0E-08	5.0	10.0	2.5	3.3
242625_at	RSAD2	Radical S-adenosyl methionine domain containing 2	ion transport	1.0E-08	5.0	10.0	2.5	5.0
222793_at	DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 (RIG-I)	innate immune response	1.0E-08	3.3	5.0	2.0	3.3
205483_s_at	G1P2	Interferon, alpha-inducible protein (clone IFI-15K)	ubiquitin cycle	1.0E-08	3.3	10.0	2.0	5.0
204415_at	G1P3	Interferon, alpha-inducible protein (clone IFI-6-16)	ubiquitin cycle	1.0E-08	3.3	5.0	2.0	2.5
219863_at	HERC5	Hect domain and RLD 5	ISG15 protein ligase	1.0E-08	3.3	10.0	2.0	3.3
219352_at	HERC6	Hect domain and RLD 6	ubiquitin ligases	1.0E-08	3.3	10.0	2.0	3.3
214059_at	IFI44	Interferon-induced protein 44	immune response	1.0E-07	3.3	10.0	2.0	2.5
203153_at	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1	immune response	1.0E-08	3.3	10.0	2.5	3.3
217502_at	IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	negative regulation of cell proliferatic	1.0E-08	3.3	10.0	2.5	3.3
201601_x_at	IFITM1	Interferon induced transmembrane protein 1 (9-27)	ubiquitin cycle	4.0E-07	3.3	5.0	2.5	2.5
219684_at	IFRG28	28kD interferon responsive protein	RNA catabolic process	1.0E-08	3.3	5.0	2.5	3.3
204994_at	MX2	Myxovirus (influenza virus) resistance 2	antiviral immune response	1.0E-08	3.3	10.0	3.3	3.3
202869_at	OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa	antiviral immune response	1.0E-08	3.3	10.0	2.5	2.5
204972_at	OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	antiviral immune response	1.0E-08	3.3	10.0	2.0	2.5
218400_at	OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	antiviral immune response	1.0E-08	3.3	10.0	2.0	2.5
228531_at	SAMD9	Sterile alpha motif domain containing 9	cell differentiation	1.0E-08	3.3	5.0	2.5	2.5
219211_at	USP18	Ubiquitin specific peptidase 18	ubiquitin cycle	1.0E-08	3.3	5.0	2.5	3.3
242234_at	BIRC4BP	XIAP associated factor-1	NF-kappaB cascade	1.2E-06	2.5	5.0	1.7	2.0
204211_x_at	EIF2AK2	Eukaryotic translation initiation factor 2-alpha kinase 2	translation	1.0E-08	2.5	5.0	1.7	2.0
227609_at	EPSTI1	Epithelial stromal interaction 1 (breast)	expressed in breast cancer	1.0E-08	2.5	5.0	2.0	2.0
211267_at	HESX1	Homeo box (expressed in ES cells) 1	development	1.0E-08	2.5	2.5	2.5	2.0
207156_at	HIST1H2AG	Histone 1, H2ag	chromosome organization	1.0E-08	2.5	2.5	2.0	2.5
202411_at	IFI27	Interferon, alpha-inducible protein 27	anti-apoptosis	3.2E-05	2.5	2.5	2.0	3.3
203596_s_at	IFIT5	Interferon-induced protein with tetratricopeptide repeats 5	transcription	1.0E-08	2.5	3.3	1.7	2.5
236156_at	LIPA	Lipase A, lysosomal acid, cholesterol esterase	protein amino acid phosphorylation	1.2E-06	2.5	5.0	2.0	3.3
209035_at	MDK	Midkine (neurite growth-promoting factor 2)	cell proliferation	8.2E-06	2.5	2.5	2.0	2.5
217546_at	MT1M	metallothionein 1M	metal ion binding	5.1E-06	2.5	2.0	2.0	3.3
223298_s_at	NT5C3	5'-nucleotidase, cytosolic III	antiviral immune response	1.0E-08	2.5	3.3	2.5	2.5
244315_at	PLSCR1	Phospholipid scramblase 1	protein phosphatase	4.4E-05	2.5	5.0	1.7	2.0
228230_at	PRIC285	Peroxisomal proliferator-activated receptor A interacting complex 285	coactivator for PPARA and PPARG	1.0E-08	2.5	5.0	1.7	2.0
226603_at	SAMD9L	Sterile alpha motif domain containing 9-like	cell differentiation	1.0E-08	2.5	5.0	2.0	2.0
208012_x_at	SP110	SP110 nuclear body protein	antiviral response	1.0E-08	2.5	3.3	2.0	2.0
206271_at	TLR3	Toll-like receptor 3	innate immune response	1.7E-05	2.5	2.5	2.0	2.5
34689_at	TREX1	Three prime repair exonuclease 1	DNA binding	7.1E-06	2.5	3.3	2.0	1.3
208087_s_at	ZBP1	Z-DNA binding protein 1	innate immune response	1.4E-05	2.5	3.3	2.5	2.0
205098_at	CCR1	Chemokine (C-C motif) receptor 1	immune response	9.9E-06	2.0	2.5	2.5	1.7
208912_s_at	CNP	2',3'-cyclic nucleotide 3' phosphodiesterase	innate immune response	1.0E-08	2.0	2.5	2.0	2.0
233660_at	EHD4	EH-domain containing 4	apoptosis	2.0E-05	2.0	3.3	2.0	1.3
224973_at	FAM46A	Family with sequence similarity 46, member A	chemotaxis	3.0E-07	2.0	2.5	1.7	2.0
214560_at	FPRL2	Formyl peptide receptor-like 2	nucleotide metabolism	6.0E-07	2.0	1.7	2.0	2.0
204187_at	GMPR	Guanosine monophosphate reductase	development	1.0E-07	2.0	2.5	2.5	1.4
215071_s_at	HIST1H2AC	Histone 1, H2ac	chromosome organization	6.4E-06	2.0	1.7	2.0	2.0
210387_at	HIST1H2BG	Histone 1, H2bg	chromosome organization	4.3E-06	2.0	2.0	1.7	2.5
206332_s_at	IFI16	Interferon, gamma-inducible protein 16	immune response	1.0E-08	2.0	2.5	2.0	1.7
209417_s_at	IFI35	Interferon-induced protein 35	immune response	3.2E-06	2.0	3.3	1.7	2.0
33304_at	ISG20	Interferon stimulated exonuclease gene 20kDa	signal transduction	1.8E-06	2.0	3.3	1.7	1.7
200923_at	LGALS3BP	Lectin, galactoside-binding, soluble, 3 binding protein	cell adhesion	1.0E-07	2.0	2.0	2.0	2.5
212713_at	MFAP4	Microfibrillar-associated protein 4	metal ion binding	2.1E-05	2.0	2.5	2.5	1.7
217165_x_at	MT1F	Metallothionein 1F	metal ion binding	6.7E-06	2.0	2.0	2.0	3.3
204745_x_at	MT1G	Metallothionein 1G	metal ion binding	4.7E-05	2.0	1.7	1.7	3.3
206963_s_at	NAT8	N-acetyltransferase 8	nucleotide metabolism	3.5E-05	2.0	2.0	2.0	2.0
225344_at	NCOA7	Nuclear receptor coactivator 7	immune response	1.0E-07	2.0	2.5	1.7	2.0
210797_s_at	OASL	2'-5'-oligoadenylate synthetase-like	antiviral immune response	4.2E-05	2.0	3.3	1.4	2.0
218543_s_at	PARP12	Poly (ADP-ribose) polymerase family, member 12	DNA repair	1.0E-08	2.0	3.3	1.7	2.0
223220_s_at	PARP9	Poly (ADP-ribose) polymerase family, member 9	DNA repair	4.0E-07	2.0	2.5	1.7	2.0
209640_at	PML	Promyelocytic leukemia	transcription	1.0E-07	2.0	2.0	2.0	2.0
225291_at	PNPT1	Polyribonucleotide nucleotidyltransferase 1	positive regulation of cell proliferatior	1.0E-08	2.0	3.3	1.7	2.0
235061_at	PPM1K	Protein phosphatase 1K (PP2C domain containing)	regulation of Rab GTPase activity	1.0E-08	2.0	2.5	1.7	2.0
237105_at	PRKRA	Protein kinase, interferon-inducible double stranded RNA (PKR)	antiviral immune response	3.0E-07	2.0	2.5	2.0	1.7
238743_at	RIN2	Ras and Rab interactor 2	NF-kappaB cascade	1.0E-08	2.0	3.3	2.0	1.4
211456_x_at	RPL35	Similar to 60S ribosomal protein L35	inflammatory response	3.1E-05	2.0	1.7	2.0	3.3
1553851_at	SPIC	Spi-C transcription factor (Spi-1/PU.1 related)	NF-kappaB cascade	1.5E-05	2.0	1.7	2.0	2.5
AFFX-HUMISGF3A/M97935_MA_at	STAT1	Signal transducer and activator of transcription 1, 91kDa	antiviral immune response	2.4E-06	2.0	3.3	1.4	1.4

213361_at	TDRD7	Tudor domain containing 7	DNA binding	1.0E-08	2.0	3.3	2.0	2.0
214329_x_at	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	inflammatory response	1.0E-07	2.0	2.5	1.7	2.0
204804_at	TRIM21	Tripartite motif-containing 21	NF-kappaB cascade	1.0E-08	2.0	3.3	2.0	1.7
223830_s_at	TRIM5	Tripartite motif-containing 5	DNA binding	6.0E-07	2.0	2.0	1.7	2.0
223599_at	TRIM6	Tripartite motif-containing 6	DNA binding	1.0E-07	2.0	2.5	1.4	2.5
219716_at	APOL6	Apolipoprotein L, 6	lipid transport	6.5E-06	1.7	2.5	1.3	1.4
201641_at	BST2	Bone marrow stromal cell antigen 2	regulation of apoptosis	5.3E-05	1.7	2.0	1.4	2.0
205467_at	CASP10	Caspase 10, apoptosis-related cysteine peptidase	regulation of apoptosis	2.0E-07	1.7	2.0	1.7	1.3
223834_at	CD274	CD274 antigen	cyclic nucleotide catabolic process	3.4E-05	1.7	1.7	1.7	1.7
225415_at	DTX3L	Deltex 3-like (Drosophila)	calcium ion binding	1.0E-08	1.7	2.5	1.4	2.0
209911_x_at	HIST1H2BD	Histone 1, H2bd	chromosome organization	3.9E-05	1.7	1.4	2.0	2.0
209806_at	HIST1H2BK	Histone 1, H2bk	chromosome organization	3.4E-05	1.7	1.4	1.4	2.5
232035_at	HIST1H4H	Histone 1, H4h	chromosome organization	2.3E-05	1.7	2.0	1.4	2.0
230795_at	HIST2H4	Histone 2, H4	chromosome organization	3.0E-07	1.7	1.7	1.7	2.0
212657_s_at	IL1RN	Interleukin 1 receptor antagonist	cell adhesion	7.0E-07	1.7	2.0	1.7	1.4
208436_s_at	IRF7	Interferon regulatory factor 7	innate immune response	5.0E-07	1.7	2.5	1.4	1.7
243999_at	MGC19764	Likely ortholog of mouse schlafen 5	metal ion binding	3.4E-05	1.7	2.5	1.4	1.4
238025_at	MLKL	Mixed lineage kinase domain-like	metal ion binding	5.4E-05	1.7	2.5	1.7	1.3
216336_x_at	MT1K	Metallothionein 1M	metal ion binding	6.3E-06	1.7	1.7	1.7	2.5
203964_at	NMI	N-myc (and STAT) interactor	nucleotide metabolism	1.0E-07	1.7	2.0	1.7	1.7
224701_at	PARP14	Poly (ADP-ribose) polymerase family, member 14	DNA repair	1.8E-06	1.7	2.5	1.7	1.4
212660_at	PHF15	PHD finger protein 15	RNA processing	6.8E-05	1.7	2.0	1.7	1.4
211737_x_at	PTN	Pleiotrophin	neurite outgrowth-promoting proteins	9.5E-06	1.7	1.7	1.7	2.0
213982_s_at	RABGAP1L	RAB GTPase activating protein 1-like	expressed in esophageal t cancer	7.4E-06	1.7	2.5	1.7	1.4
222986_s_at	SCOTIN	Scotin	regulation of apoptosis	4.0E-06	1.7	2.0	1.4	2.0
223192_at	SLC25A28	Solute carrier family 25, member 28	steroid biosynthetic process	1.0E-07	1.7	2.5	1.4	1.4
219519_s_at	SN	Sialoadhesin	humoral immune responses	2.4E-06	1.7	2.0	1.7	1.4
226390_at	STARD4	START domain containing 4, sterol regulated	metabolism of cholesterol	3.8E-05	1.7	2.0	1.4	1.7
213293_s_at	TRIM22	Tripartite motif-containing 22	antiviral response	1.3E-05	1.7	2.5	1.3	1.4
224806_at	TRIM25	Tripartite motif-containing 25	ubiquitin cycle	3.0E-07	1.7	2.5	1.7	1.4
221044_s_at	TRIM34	Tripartite motif-containing 34	ubiquitin cycle	1.0E-08	1.7	1.7	1.7	1.4
203610_s_at	TRIM38	Tripartite motif-containing 38	zinc ion binding	1.0E-08	1.7	2.0	1.7	1.4
219062_s_at	ZCCHC2	Zinc finger, CCHC domain containing 2	DNA binding	1.0E-08	1.7	2.5	1.7	1.7

genotype 1-Rsp: Patients infected with genotype 1 HCV with SVR or TR
genotype 1-nonRsp: Patients infected with genotype 1 HCV with NR
genotype 2: Patients infected with genotype 2 HCV

Supplementary Table 2-1. Representative genes specifically expressed in cells in liver lobules

Probe Set	Symbol	Description	CLL/CPA	*p* -value
217558_at	CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide	23.5	< 1e-07
207608_x_at	CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide	21.5	2.00E-06
206054_at	KNG1	Kininogen 1	21.2	1.00E-07
219466_s_at	APOA2	Apolipoprotein A-II	18.8	1.00E-06
231398_at	SLC22A7	Solute carrier family 22, member 7	18.3	1.00E-07
203400_s_at	TF	Transferrin	17.4	2.80E-06
1431_at	CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide	17.3	9.00E-07
205216_s_at	APOH	Apolipoprotein H	16.9	8.00E-07
39763_at	HPX	Hemopexin	16.8	1.10E-06
222049_s_at	RBP4	Retinol binding protein 4, plasma	16.5	3.00E-07
210049_at	SERPINC1	Serpin peptidase inhibitor, clade C, member 1	16.0	6.20E-06
206305_s_at	C8A	Complement component 8, alpha polypeptide	14.8	1.00E-07
217238_s_at	ALDOB	Aldolase B, fructose-bisphosphate	14.7	3.00E-07
206754_s_at	CYP2B7P1	Cytochrome P450, family 2, subfamily B, polypeptide	14.3	7.30E-06
206753_at	RDH16	Retinol dehydrogenase 16 (all-trans and 13-cis)	14.2	< 1e-07
207414_s_at	PCSK6	Proprotein convertase subtilisin/kexin type 6	14.0	1.00E-07
205108_s_at	APOB	Apolipoprotein B	14.0	6.00E-07
206119_at	BHMT	Betaine-homocysteine methyltransferase	13.7	1.00E-07
206386_at	SERPINA7	Serpin peptidase inhibitor, clade A, member 7	13.4	1.20E-06
208470_s_at	HP	Haptoglobin	13.3	3.00E-07
220383_at	ABCG5	ATP-binding cassette, sub-family G, member 5	13.1	5.10E-06
204988_at	FGB	Fibrinogen beta chain	13.0	2.39E-05
207820_at	ADH1A	Alcohol dehydrogenase 1A, alpha polypeptide	13.0	3.00E-07
204450_x_at	APOA1	Apolipoprotein A-I	12.8	8.00E-07
205813_s_at	MAT1A	Methionine adenosyltransferase I, alpha	12.7	4.00E-07
206840_at	AFM	Afamin	12.4	9.00E-07
1554837_a_a	CYP4A11	Cytochrome P450, family 4, subfamily A, polypeptide	12.4	8.00E-07
207041_at	MASP2	Mannan-binding lectin serine peptidase 2	11.8	1.00E-07
209613_s_at	ADH1B	Alcohol dehydrogenase IB, beta polypeptide	11.6	1.00E-07
208147_s_at	CYP2C8	Cytochrome P450, family 2, subfamily C, polypeptide	11.5	4.00E-07
216025_x_at	CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide	11.5	< 1e-07
220224_at	HAO1	Hydroxyacid oxidase (glycolate oxidase) 1	11.5	8.00E-07
207202_s_at	NR1I2	Nuclear receptor subfamily 1, group I, member 2	11.4	2.60E-06
207218_at	F9	Coagulation factor IX	11.4	2.00E-06
1554459_s_a	CFH	Complement factor H	11.1	6.00E-07
205871_at	PLGLB2	Plasminogen-like B2	11.0	< 1e-07
206292_s_at	SULT2A1	Sulfotransferase family, cytosolic, 2A	11.0	9.00E-07
206065_s_at	DPYS	Dihydropyrimidinase	10.9	2.00E-07
223781_x_at	ADH4	Alcohol dehydrogenase 4 (class II), pi polypeptide	10.9	< 1e-07
211298_s_at	ALB	Albumin	10.8	2.57E-05

Supplementary Table 2-2. Representative genes specifically expressed in cells in portal areas

Probe Set	Symbol	Description	CPA/CLL	*p* -value
217028_at	CXCR4	Chemokine (C-X-C motif) receptor 4	27.4	1.00E-07
1563674_at	FCRL2	Fc receptor-like 2	22.9	< 1e-07
226218_at	IL7R	Interleukin 7 receptor	20.6	< 1e-07
236341_at	CTLA4	Cytotoxic T-lymphocyte-associated protein 4	16.3	2.00E-06
233813_at	PPP1R16B	Protein phosphatase 1, regulatory (inhibitor) subunit 16B	15.7	3.20E-06
1560396_at	KLHL6	Kelch-like 6 (Drosophila)	15.1	2.90E-05
230983_at	BCNP1	B-cell novel protein 1	14.4	7.00E-07
213790_at	ADAM12	ADAM metallopeptidase domain 12 (meltrin alpha)	13.8	9.72E-05
207564_x_at	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase	13.7	3.02E-05
1559078_at	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	13.1	2.23E-05
231093_at	FCRH3	Fc receptor-like 3	13.0	3.00E-06
209771_x_at	CD24	CD24 antigen	12.9	3.00E-07
230110_at	MCOLN2	Mucolipin 2	12.7	1.41E-05
230894_s_at	MSI2	Musashi homolog 2 (Drosophila)	12.2	6.49E-05
209480_at	HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	12.1	4.60E-06
232406_at	JAG1	Jagged 1 (Alagille syndrome)	11.6	1.74E-05
224406_s_at	FCRL5	Fc receptor-like 5	11.1	2.00E-07
212588_at	PTPRC	Protein tyrosine phosphatase, receptor type, C	10.8	4.90E-06
204794_at	DUSP2	Dual specificity phosphatase 2	10.4	2.57E-05
213888_s_at	TRAF3IP3	TRAF3 interacting protein 3	10.3	8.00E-07
213891_s_at	TCF4	Transcription factor 4	10.2	2.00E-07
213603_s_at	RAC2	Ras-related C3 botulinum toxin substrate 2	9.9	< 1e-07
204563_at	SELL	Selectin L	9.8	5.00E-07
229686_at	P2RY8	Purinergic receptor P2Y, G-protein coupled, 8	9.6	8.00E-07
236295_s_at	NOD3	NOD3 protein	9.6	7.50E-06
225598_at	SLC45A4	Solute carrier family 45, member 4	9.4	8.00E-07
225763_at	RCSD1	RCSD domain containing 1	9.3	< 1e-07
203936_s_at	MMP9	Matrix metallopeptidase 9	9.0	9.00E-07
202336_s_at	PAM	Peptidylglycine alpha-amidating monooxygenase	8.6	1.09E-05
201721_s_at	LAPTM5	Lysosomal associated multispanning membrane protein 5	8.5	< 1e-07
204440_at	CD83	CD83 antigen	8.5	6.00E-06
213193_x_at	TRBV19	T cell receptor beta variable 19	8.5	< 1e-07
209828_s_at	IL16	Interleukin 16	8.3	6.00E-06
205590_at	RASGRP1	RAS guanyl releasing protein 1	8.3	4.48E-05
203868_s_at	VCAM1	Vascular cell adhesion molecule 1	8.1	8.80E-06
202992_at	C7	Complement component 7	8.0	1.13E-05
210072_at	CCL19	Chemokine (C-C motif) ligand 19	8.0	5.00E-07
214247_s_at	DKK3	Dickkopf homolog 3	8.0	1.40E-06
210448_s_at	P2RX5	Purinergic receptor P2X, ligand-gated ion channel, 5	8.0	2.81E-05
202499_s_at	SLC2A3	Solute carrier family 2, member 3	7.9	2.33E-05

Supplementary Table 3. Expression of genes responsible for the innate immune response to IFN and IFN resistance in genotype 1 patients

ID	Symbol	Gene Name	IFN induction (During therapy/Before therapy)					
			CLL		CPA		Whole liver	
			Rsp	nonRsp	Rsp	nonRsp	Rsp	nonRsp
		Innate immune response genes						
222793_at	DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	**6.2***	1.3	**2.5***	1.9	**5.0***	**2.0***
219209_at	IFIH1	Interferon induced with helicase C domain 1 (MDA5)	**3.8***	1.1	**5.1***	**2.5***	**5.0***	**2.5***
231829_at	KIAA1271	KIAA1271 protein (MAVS)	ND	ND	0.8	1.8	0.9	1.3
239876_at	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells	1.3	1.2	1.0	1.9	0.8	0.7
204533_at	CXCL10	Chemokine (C-X-C motif) ligand 10	**3.4***	0.6	**7.7***	1.5	**2.0***	1.0
208436_s_at	IRF7	Interferon regulatory factor 7	1.4	1.3	**2.7***	**2.4***	**2.5***	**1.4***
218520_at	TBK1	TANK-binding kinase 1	**2.5***	1.0	2.4	1.5	1.3	1.3
238494_at	TRAF3IP1	TNF receptor-associated factor 3 interacting protein 1	1.9	0.7	0.4	0.5	1.0	1.1
205558_at	TRAF6	TNF receptor-associated factor 6	**2.6***	0.9	0.5	**0.5***	1.0	1.1
213191_at	TICAM1	Toll-like receptor adaptor molecule 1	1.2	1.0	1.0	0.6	1.3	1.1
209941_at	RIPK1	Receptor (TNFRSF)-interacting serine-threonine kinase 1	1.6	**0.3***	4.0	1.0	**1.3***	1.4
		Inhibitory genes for IFN signaling						
228895_s_at	ASB1	Ankyrin repeat and SOCS box-containing 1	0.7	**3.2***	1.4	1.2	0.8	0.7
1563505_at	DUSP16	Dual specificity phosphatase 16	0.5	**3.5***	**0.5***	**0.4***	0.8	0.8
225452_at	PPARBP	PPAR binding protein	1.0	**4.5***	2.2	1.2	0.8	1.1
209685_s_at	PRKCB1	Protein kinase C, beta 1	0.6	**3.4***	1.2	0.7	0.9	1.1
206687_s_at	SHP1	Protein tyrosine phosphatase, non-receptor type 6	0.8	0.8	0.9	0.7	0.9	1.0
205128_x_at	PTGS1	Prostaglandin-endoperoxide synthase 1	1.4	**3.5***	1.4	0.9	0.6	1.3
211000_s_at	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	1.3	**3.0***	2.0	1.7	1.3	**1.4***
210479_s_at	RORA	RAR-related orphan receptor A	0.7	**3.5***	0.6	1.6	1.0	1.0
228070_at	PPP2R5E	Protein phosphatase 2, regulatory subunit B (B56), epsilon isoform	1.3	**3.0***	1.1	1.1	**1.4***	**1.3***
206360_s_at	SOCS3	Suppressor of cytokine signaling 3	0.8	**2.3***	1.1	0.7	1.1	1.0
217864_s_at	PIAS1	Protein inhibitor of activated STAT-1	1.5	**1.9***	0.8	1.4	1.1	1.1
203927_at	NFKBIE	NFKB inhibitor epsilon	0.4	**2.0***	1.3	1.5	1.0	1.1

Rsp: viral responder; patients with SVR or TR
nonRsp: non-viral responder; patients with NR
CLL: cells in liver lobles
CPA: cells in portal area
NS:not significant; ND: not determined, *P<0.05

Supplemental Fig 1