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Interferon stimulated exonuclease gene 20kDa links psychiatric events to distinct Hepatitis C Virus responses in Human Immunodeficiency Virus positive patients

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Abstract

Hepatitis C Virus (HCV) infection occurs frequently in patients with preexisting mental illness. Treatment for chronic hepatitis C using interferon formulations often increases risk for neuropsychiatric symptoms. Pegylated-Interferon-α (PegIFN-α) remains crucial for attaining sustained virologic response (SVR); however, PegIFN-α based treatment is associated with psychiatric adverse effects, which require dose reduction and/or interruption. This study's main objective was to identify genes induced by PegIFN-α and expressed in the central nervous system and immune system, which could mediate the development of psychiatric toxicity in association with antiviral outcome. Using peripheral blood mononuclear cells from Human Immunodeficiency Virus (HIV)/HCV co-infected donors (N=28), DNA microarray analysis was performed and 21 differentially regulated genes were identified in patients with psychiatric toxicity vs. those without. Using these 21 expression profiles a two-way-ANOVA was performed to select genes based on antiviral outcome and occurrence of neuropsychiatric adverse events. Microarray analysis demonstrated that Interferon-stimulated-exonuclease-gene 20kDa (ISG20) and Interferon-alpha-inducible-protein 27 (IFI27) were the most regulated genes ($P<0.05$) between three groups that were built by combining antiviral outcome and neuropsychiatric toxicity. Validation by bDNA assay confirmed that ISG20 expression levels were significantly associated with these

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outcomes (P<0.035). Baseline levels and induction of ISG20 correlated independently with no occurrence of psychiatric adverse events and non-response to therapy (P<0.001). Among the 21 genes that were associated with psychiatric adverse events and 20 Interferon-inducible genes (IFIGs) used as controls, only ISG20 expression was able to link PegIFN-α related neuropsychiatric toxicity to distinct HCV-responses in patients co-infected with HIV and HCV *in vivo*.

Keywords

HIV/HCV co-infection; Interferon-α; Neuropsychiatric Toxicity; ISG20

Introduction

Among 40 million people worldwide infected with the Human Immunodeficiency Virus (HIV), approximately 5 million are also co-infected chronically with the hepatitis C Virus (HCV) [Alter, 2006]. To date, treatment with pegylated Interferon-α (PegIFN-α) in combination with ribavirin (RBV) has been proven to achieve sustained virologic response (SVR) in up to 40% of all treated patients. However, PegIFN-α/RBV triggers the development of neuropsychiatric adverse effects including anxiety, irritability, mood lability, depression and suicidal behavior [Laguno et al., 2004; Weiss and Gorman, 2006]. Psychiatric toxicity occurs within the first few months of treatment and may affect up to 50% of all patients receiving PegIFN-α based therapy [Sulkowski and Thomas, 2005]. Such severe adverse events represent a frequent reason for dose reduction or treatment discontinuation [Zdilar et al., 2000], especially in patients co-infected with HIV and HCV, who exhibit higher rates of neuropsychiatric illness relative to HIV mono-infected persons or the general population [Goulet et al., 2005; Weiss and Gorman, 2006].

Previously, a diagnostic biomarker panel has been described as able to predict emergent psychiatric toxicity in patients co-infected with HIV and HCV undergoing PegIFN-α/RBV treatment [Rasimas et al., 2012]. This study was aimed to further explore the molecular basis of the clinical outcome observed in patients who experience a psychiatric toxicity and achieve sustained virologic response) [Rasimas et al., 2012], similar to findings published using HCV mono-infected patients [Loftis et al., 2004]. To date, it is unclear if psychiatric adverse events are more commonly associated with a favorable therapeutic outcome. If this is correct, then proper management of these adverse events and continued therapy is critical to obtain optimal sustained virologic response rates in these patients. Furthermore, it is also not clear whether the molecular mechanisms involved in pathogenesis of PegIFN-α associated mood disorders and those involved in permanent viral clearance are necessarily the same. Although direct acting antiviral therapies have been developed to offer a viable Interferon-free regimen, PegIFN-α remains the backbone of present treatment for chronic hepatitis C at the moment. In this regard, this study was aimed to discover novel molecular targets/mechanisms that govern viral response and neuropsychiatric toxicity in patients co-infected with HIV and HCV receiving PegIFN-α based treatment. To this end, emergence of psychiatric symptoms like depression, mood lability, anxiety or psychotic behavior was evaluated as detailed elsewhere [Rasimas et al., 2012].

This study is distinct from previous work [Rasimas et al., 2012] as the primary focus has been put on specific genes/pathways that are enriched in immune cells and CNS cells. Here, DNA microarray analysis has been performed using peripheral blood mononuclear cells (PBMCs) obtained from patients co-infected with HIV and HCV before and after treatment with PegIFN-α/RBV and a four-step annotation algorithm resulted in the identification of two molecular markers significantly involved in the occurrence of sustained virologic response and psychiatric toxicity, i.e., Interferon stimulated exonuclease gene 20kDa (ISG20) and Interferon alpha-inducible protein 27 (IFI27). These findings provide yet another evidence in support of the hypothesis that gene regulation in peripheral immune cells might be connected causally to neuropsychiatric events in the brain, since it has been shown that HIV infection is associated with disruption of the blood-brain-barrier and increased leukocyte penetration into the CNS [Lafrenie et al., 1997; Persidsky et al., 2000].

Materials and Methods

Study subjects

Patients (N=32) with stable HIV disease and chronic HCV infection were enrolled in Institutional Review Board approved studies at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland for treatment of HCV infection with pegylated Interferon-α2b (1.5μg/Kg/wk) and ribavirin 1000-1200mg/day. Four patients were taken off study: three were lost to follow up and one dropped out for social reasons after only one dose of PegIFN-α.

Ethics statement—All patients signed an Institutional Review Board approved protocol informed consent document.

Study inclusion and exclusion criteria

Patients co-infected with HIV and HCV were eligible if they were older than 18 years, had a CD4 count >100 cells/mm^3, HCV viral load >2000 copies/ml, had histological evidence of chronic HCV infection and stable HIV disease being managed according to current HIV treatment guidelines. Participants with active psychiatric illness, i.e. mood lability, anxiety, or psychotic symptoms, had been treated and stabilized prior to enrollment; 3 patients got new prescriptions for citalopram, 1 was prescribed a higher dose of escitalopram, 1 was given risperidone and 1 maintained mirtazapine throughout the anti-HCV treatment as reported previously [Rasimas et al., 2012]. Four patients who participated in this study (including two of those given citalopram) increased their frequency of psychotherapy visits before study entry [Rasimas et al., 2012].

Psychiatric evaluations

All patients underwent standard pretreatment psychiatric evaluations conducted by board-certified psychiatrists from the National Institute of Mental Health as noted elsewhere [Rasimas et al., 2012]. The clinical assessment of mood stability was corroborated by Beck Depression Inventory (BDI)-II scores of less than or equal to 9 at the time of PegIFN-α/RBV treatment initiation [Rasimas et al., 2012]. Patients (N=28) who received at least 4 weeks of PegIFN-α/RBV treatment were classified into two groups, i.e., those with

psychiatric toxicity and those without, after initiation of therapy in a blinded fashion prior to gene expression analysis as stated previously [Rasimas et al., 2012].

Microarray analysis

PBMCs from patients co-infected with HIV and HCV (N=28) were obtained at baseline and post treatment (within five days after last administration of PegIFN-α/RBV), and were subjected to DNA microarray analysis (Affymetrix HG-U133A) as described previously [Lempicki et al., 2006]. Post treatment, 3 out of 28 samples were excluded from the analysis as they failed chip quality control tests. Data analysis was carried out in four steps. *STEP ONE:* one-way-ANOVA (PARTEK Genomics Suite) was performed based on two outcome parameters: presence or absence of psychiatric toxicity. A Mean Fold Difference (MFD) representing the absolute mean difference in gene expression ($\log_2$) between both groups (patients with or without psychiatric toxicity) was calculated for selected genes at two different time points, i.e, pre and post treatment. Furthermore, a Mean Fold Change (MFC) was calculated for every gene within each group as the absolute mean difference in gene expression ($\log_2$) between two different time points (pre versus post treatment). This statistical approach is unique from the one that was employed in previous work [Rasimas et al., 2012], which was aimed exclusively to identify the genes that predict emergence of neuropsychiatric toxicity. For all analyses in the present study, a P-value of <0.05, an absolute $\log_2$MFD of >0.38 and an absolute $\log_2$MFC of >0.38 were considered significant. Subsequently, sixteen gene subsets were identified that passed these strict cutoffs for significance. *STEP TWO:* Venn diagrams were assembled between these sixteen gene subsets in order to identify expression profiles with consistently significant modulation in at least two of the categories (psychiatric toxicity, sustained virologic response and or induction by therapy). *STEP THREE:* Further selection was based on gene characterization using the functional annotation tool DAVID [Huang da et al., 2009]. Finally, four gene subsets (including twenty two probe IDs) involving 21 unique genes reached statistical significance based on the above criteria and exhibited significant enrichment in brain tissue or were associated with neuropsychiatric disorders based on DAVID [Huang da et al., 2009]. There were five genes that were common to previous work [Rasimas et al., 2012] (Supplementary Object 1). *STEP FOUR:* Using expression data of those 21 unique genes, two-way-ANOVA was performed based on pair-wise comparisons between three groups: patients with psychiatric toxicity who reached sustained virological response (N=8, 28%) vs. patients with psychiatric toxicity who did not (N=10, 36%) vs. patients who remained uneventful non-responders (N=10, 36%). There were no patients who achieved sustained virologic response but did not experience a psychiatric adverse event in this study. This could be a random epidemiological outcome and lacks any other reasonable explanation for this observation. Selected results were validated by a custom bDNA assay 1.0 for IFIGs.

QuantiGene-Plex Assay (QGP 1.0)

Validation of DNA microarray data was performed using bDNA assay capable of detecting expression levels of 20 genes. This 20-plex IFIGs panel was custom-ordered from Panomics, Inc. (Fremont, CA; http://www.panomics.com/index.php?id=qgpsc&page=5). PBMCs from all patients at both time points were pelleted and lysed in 2:1 PBS and

Panomics' Lysis Mixture. The assay was performed following the manufactures' protocol as described elsewhere [Kottilil et al., 2009].

Results

Overall, 18 patients co-infected with HIV and HCV developed psychiatric adverse events during treatment with PegIFN-α/RBV, while 10 patients did not. Baseline characteristics of patients who developed toxicity and those who did not were similar with regards to age, sex, race, Body Mass Index, HCV genotype, HCV viral load, $CD4^+$ T-cell counts, HIV viral load, highly active antiretroviral therapy (HAART), underlying psychiatric history and psychiatric medication prior to enrollment. (For details please see under Materials and Methods → Study inclusion and exclusion criteria and elsewhere [Rasimas et al., 2012]). In this study, the incidence of PegIFN-α induced depression (29%) was lower than reported by other investigators [Laguno et al., 2004].

Using a combined statistical and functional characterization algorithm as described in the Materials and Materials section a total of 21 unique genes were selected as regulated significantly in at least two outcome based analyses. A supervised clustering (heat map I-IV) was performed after mean-shift normalization of the data in order to highlight expression differences between these genes pre and post therapy (Figure 1). Based on DAVID, all genes included in heat map I [Anikster et al., 2002; Iwamoto et al., 2004; Millar et al., 2005], heat map III [Gardner and Ghorpade, 2003; Labrada et al., 2002; Maisel et al., 2007; Sakamoto et al., 2007] and heat map IV [Beasley et al., 2005; Cicin-Sain et al., 2008; Han et al., 2007; Jentsch, 1992; Le-Niculescu et al., 2009; Simmen et al., 2008; Wegner et al., 2008; Wu, 2006; Zhang et al., 2004] were enriched significantly in various brain tissues ($P_I<0.03$; $P_{III}<0.02$; $P_{IV}<0.01$), while all genes included in heat map II [Ainiala et al., 2004; Georgieva et al., 2008; Hamilton et al., 2004; Millar et al., 2005; Skibinski et al., 2005; Spleiss et al., 1998] were associated significantly with a number of neuropsychiatric symptoms or syndromes ($P_{II}<0.01$) (Figure 1). Interestingly, 11 (52%) out of 21 selected genes were enriched significantly in CD4+ T-cells ($P<0.025$) according to DAVID (Supplementary Object 2).

The objective of this study was to extend previous observations of a significant correlation between a sustained virologic response and the occurrence of psychiatric adverse events ($P<0.025$) and further elucidate the molecular mechanisms involved in the *in vivo* interaction between PegIFN-α induced viral response and neuropsychiatric toxicity. In this regard, two-way-ANOVA was performed after assigning patients into three groups, those who achieved sustained virologic response and had psychiatric toxicity (N=8) vs. those who did not achieve sustained virologic response but had psychiatric toxicity (N=10) vs. those who did not achieve sustained virologic response nor had toxicity (N=10), using expression values of 21 unique genes in three different settings: pre (baseline), post (end of treatment), and pre-to-post (induced) PegIFN-α/RBV therapy. Pre treatment: Looking for expression profiles that had an ANOVA P-value (P_A) of <0.05 and were regulated significantly ($P<0.05$ and absolute $\log_2 MFD>0.38$) in at least two contrasts, only 2 genes could be selected: ISG20 (Figure 2A, Figure 2C) and IFI27 (Supplementary Object 3A). Pre-to-post treatment: Interestingly, ISG20 and IFI27 were the only genes that showed a P_A of <0.05 and were

induced significantly ($P<0.05$ and absolute $\log_2$MFC>0.38) in at least two groups: patients who had psychiatric toxicity with or without sustained virologic response. In those who had neither sustained virologic response nor toxicity, only IFI27 was induced significantly ($P<0.0001$; absolute $\log_2$MFC=4.6) (Supplementary Object 3A). Post treatment: Utilizing identical criteria and cut-offs as above, only the gene "lanosterol synthase (LSS)" was found as regulated significantly in the same inter-group contrasts (data not shown). Based on these findings, genes with known significant expression in the CNS such as ISG20 and IFI27 might represent novel potential biomarkers and/or regulatory molecules for the *in vivo* interaction between behavioral toxicity and virologic response during PegIFN-α/RBV therapy in HIV/HCV co-infection.

Microarray data were confirmed using the QGP 1.0 assay that detected overall significant differences in gene expression of ISG20 ($P_A<0.0002$) at baseline ($P<0.05$ and absolute $\log_2$MFD>0.38) along with a lack of induction in patients who did not achieve sustained virologic response regardless of toxicity (Figure 2B, Figure 2D). Of note, linear regression analysis showed overall consistent detection performance of ISG20 expression by two different probe sets (33304_at and 204698_at) ($P<5.8E-11$), pre and post treatment. In a further effort to delineate its functional specificity regarding CNS toxicity, gene expression of ISG20 was compared to that of nineteen other IFIGs that also had been evaluated by the QGP 1.0 assay, at baseline; of those, ISG20 was the only gene regulated significantly among non-responders who experienced a psychiatric toxicity when compared to the ones who did not (Table 1). In addition, validation of microarray data using the QGP1.0 Assay confirmed independently a significant up-regulation of ISG20 at baseline in patients who did not develop a psychiatric adverse event ($P<0.001$) and those who did not achieve sustained virological response ($P<0.0003$) (Table 2A). However, these were not observed with IFI27 expression at baseline (Supplementary Object 3B).

Discussion

This study demonstrates a significant association between occurrence of a psychiatric adverse event and a sustained virologic response in subjects co-infected with HIV and HCV and treated with PegIFN-α/RBV. Further, a novel molecular marker, ISG20, has been identified whose baseline expression is able to discriminate patients co-infected with HIV and HCV based on virologic response and psychiatric toxicity.

As previously reported, occurrence of toxicity to PegIFN-α based therapy in subjects co-infected with HIV and HCV is closely associated with achieving virologic suppression and effectiveness of therapy [Rasimas et al., 2012]. As consistent observations have been reported by other investigators in this regard [Loftis et al., 2004] a DNA microarray analysis was utilized to shed light on molecular mechanisms that could govern this crucial clinical interaction outcome. Hereby, a novel molecular marker, ISG20, could be identified and validated as capable to distinguish clearly between three different "response-toxicity" interaction outcomes.

Thus far, cumulative research data suggested that development of toxicity might serve as clinical marker for a well functioning IFN-α pathway and therefore also for effective

responses to antiviral treatment in patients co-infected with HIV and HCV [Osinusi et al.]. In addition, it has been shown previously that up-regulation of IFIGs expression at baseline is associated with a lack of response to therapy in this difficult to treat population [Lempicki et al., 2006]. This study demonstrated that ISG20 expression levels were clearly associated with occurrence of a sustained virologic response as well as occurrence of a psychiatric toxicity. In this regard, the lowest ISG20 expression levels at baseline and the strongest ISG20 induction by PegIFN-α were associated with occurrence of sustained virologic response along with psychiatric toxicity. These important observations provide a molecular basis for PegIFN-α induced neuro-psychiatric toxicity in association with sustained vilological responses in patients co-infected with HIV and HCV.

When exploring further the relevance of ISG20 regulation on CNS toxicity, evidence appeared to support the association of a robust ISG20 expression with the survival of motor neuron (SMN)-containing macromolecular nuclear complexes. While these complexes are required for biogenesis of various small nuclear ribonucleoproteins [Espert et al., 2006], a few candidates have been reported as deregulated significantly in association with major depression in microarray based studies using post mortem prefrontal cortices [Tochigi et al., 2008]. These results suggest that the loss of SMN is incompatible with life while decreased levels lead to serious inherited motor neuron diseases [Monani, 2005]. Also, accelerated SMN protein expression has been described during differentiation of human mesenchymal stem cells into functional neuron-like cells [Alexanian et al., 2008]. Hence, it is not surprising that ISG20 is enriched and expressed at high levels in neural progenitor cells (NPC) in several regions of the adult human brain such as the cortex, the hippocampus and the subventricular zone of the lateral ventricles [Maisel et al., 2007]. NPC are pluripotent stem cells that are capable to self-renew and evolve into three cell types of the central nervous system (neurons, oligodendrocytes and astrocytes) [Maisel et al., 2007]. Cellular integrity in these zones has been shown to be relevant in the pathogenesis of several mental illnesses. Specifically, decreased cell volume in the hippocampus and the subventricular zone have been documented in depression using animal models [Lau et al., 2007]. Furthermore, these anatomic changes can be reversed with serotonergic antidepressant treatment [Paizanis et al., 2007], which is effective for helping patients to complete HCV therapy when neuropsychiatric adverse events occur [Osinusi et al.]. Thus, findings from this study indicate that increased ISG20 expression levels in PBMCs from IFN-α naïve HIV/HCV co-infected individuals may serve as a molecular marker for protection against the occurrence of psychiatric adverse events. Although, the authors did not study CNS tissues, a peripheral Interferon stimulated gene (ISG) expression may be associated with high CNS expression of ISG20 and could lead to a reduced susceptibility to PegIFN-α induced neuropsychiatric toxicity. Most interestingly, ISG20 was the only gene among twenty different Interferon stimulated genes (used as controls) confirmedly capable of discriminating between the occurrence of a neuropsychiatric event among those who did not achieve an sustained virologic response, suggesting a mechanistic role for ISG20 regulation on the development of neuropsychiatric toxicity in patients co-infected with HIV and HCV.

In support of the hypothesis that PegIFN therapy can lead to induction of ISGs in the CNS, it has been shown that type I IFNs can promote directly NPC survival in mice [Hirsch et al.,

2009]. This study reiterates the fact that systemic IFN-α can gain access to and act directly within the CNS in mice and in humans [Raison et al., 2009; Wang et al., 2008].

Furthermore, there is evidence to suggest that both ISG20 and SMN proteins are expressed (inside the cell nucleus) in proximity of or overlap with coiled bodies also known as Cajal bodies [Young et al., 2001]. Although the human SMN protein is present in virtually all tissues, it is expressed at highest levels in the CNS and liver [Young et al., 2001]. An accumulation of ISG20 in Cajal bodies after IFN treatment [Espert et al., 2006], may interact with SMN proteins mediating neuropsychiatric event in the CNS along with hepatic viral clearance in patients co-infected with HIV and HCV receiving treatment with PegIFN-α/RBV.

Recent studies revealed that ISG20 encodes a 3′ to 5′ exoribonuclease member of the DEDD superfamily of exonucleases that degrades specifically single-stranded RNA [Espert et al., 2006; Zhou et al.]. When expressed at high levels *in vitro*, ISG20 was capable of restricting infections by HIV [Espert et al., 2005] and also inhibiting HCV replication [Jiang et al., 2008]. In this regard, PegIFN-α induced up-regulation of ISG20 has been shown to be associated with favorable therapeutic outcomes in several studies [Lempicki et al., 2006].

In summary, ISG20 expression is associated with IFN-α response culminating in HCV clearance (in the liver) as well as regulation of neuropsychiatric signals (in the CNS). These data may contribute to the development of individualized prevention strategies against psychiatric toxicities in patients likely to achieve sustained virologic response and could be particularly beneficial for patients co-infected with HIV and HCV as these individuals are considered especially vulnerable to depression and other mood disorders [Goulet et al., 2005; Weiss and Gorman, 2006]. Finally, these results could also be of benefit for the management of patients receiving type I IFN based therapy for other diseases such as melanoma or multiple sclerosis [Zdilar et al., 2000].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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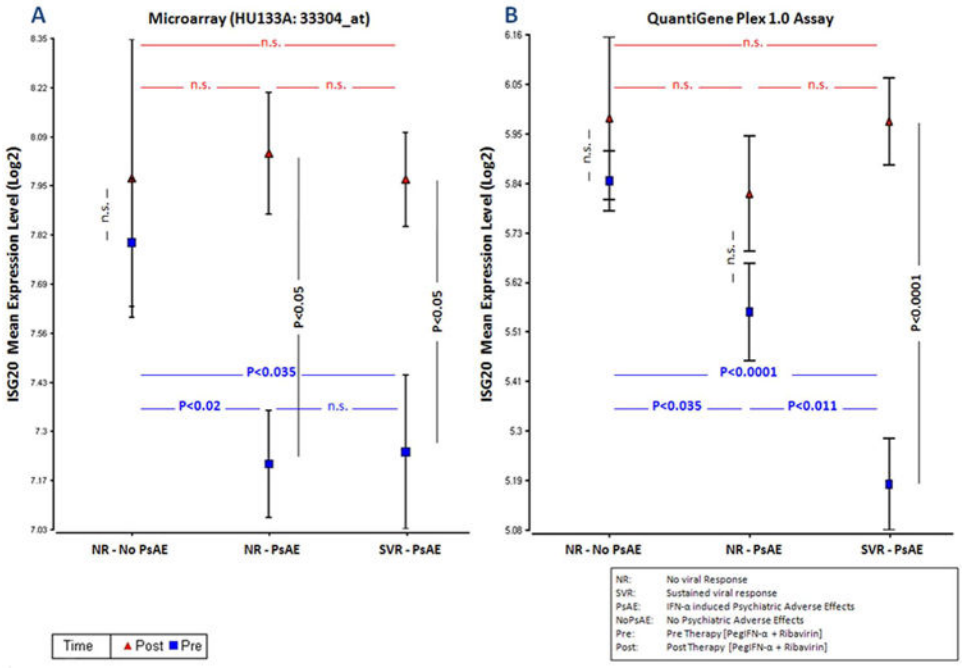
Figure 1. Expression differences of 21 genes between patients with psychiatric events (PsAE) and without (NoPsAE), pre and post therapy

In patients (N=28) co-infected with the Human Immunodeficiency Virus/Hepatitis C Virus (HIV/HCV) DNA microarray analysis was performed using peripheral blood mononuclear cells (PBMCs). Serial overlapping of selected genes based on multi-contrast statistical and functional characterization algorithms led to identification of three gene subsets (heat map I-III) representing a panel of 12 unique molecular factors that reflect different biological states with respect to the development of Interferon-α (IFN-α) associated psychiatric adverse effects (PsAE). Furthermore, a 9-gene signature (heat map IV) characterized by sustained expression differences between eventful and uneventful patients pre and post therapy with pegylated Interferon-α and ribavirin (PegIFN-α/RBV) has been identified as containing genetic imprints associated likely with HIV and/or HCV related neurobehavioral disorders.



C

Microarray Results (HU133A: 33304_at)

ISG20 (33304_at)	Contrast	P-value ANOVA
Pre	NR-NoPsAE vs. NR-PsAE	**0.018245**
Pre	NR-NoPsAE vs. SVR-PsAE	**0.033437**
Pre	NR-PsAE vs. SVR-PsAE	0.897073

ISG20 (33304_at)	Contrast	P-value ANOVA
Post	NR-NoPsAE vs. NR-PsAE	0.851018
Post	NR-NoPsAE vs. SVR-PsAE	0.994013
Post	NR-PsAE vs. SVR-PsAE	0.861254

ISG20 (33304_at)	Contrast	P-value ANOVA
NR-NoPsAE	Post vs. Pre	0.564337
NR-PsAE	Post vs. Pre	**0.006044**
SVR-PsAE	Post vs. Pre	**0.042172**

D

QuantiGene Plex 1.0 Assay Results

ISG20 (QGP1.0)	Contrast	P-value ANOVA
Pre	NR-NoPsAE vs. NR-PsAE	**0.034352**
Pre	NR-NoPsAE vs. SVR-PsAE	**3.93E-05**
Pre	NR-PsAE vs. SVR-PsAE	**0.010371**

ISG20 (QGP1.0)	Contrast	P-value ANOVA
Post	NR-NoPsAE vs. NR-PsAE	0.412464
Post	NR-NoPsAE vs. SVR-PsAE	0.973325
Post	NR-PsAE vs. SVR-PsAE	0.44689

ISG20 (QGP1.0)	Contrast	P-value ANOVA
NR-NoPsAE	Post vs. Pre	0.414486
NR-PsAE	Post vs. Pre	0.126971
SVR-PsAE	Post vs. Pre	**6.58E-05**

NR: No viral Response
SVR: Sustained viral response
PsAE: IFN-α induced Psychiatric Adverse Effects
NoPsAE: No Psychiatric Adverse Effects
Pre: Pre Therapy [PegIFN-α + Ribavirin]
Post: Post Therapy [PegIFN-α + Ribavirin]

Figure 2. Expression levels of ISG20, pre and post therapy

Microarray analysis yielded expression of Interferon stimulated exonuclease gene 20kDa (ISG20) at highest level in the group of uneventful non-responders (NR-NoPsAE) at baseline (Figure 2A). Once validated, eventful responders (SVR-PsAE) showed the lowest ISG20 expression at baseline (Figure 2B). Statistical results for all contrasts pre (baseline), pre-to-post (induction) or post therapy are shown in Figure 2C and 2D.

Table 1

Correlation between baseline expression levels of IFIGs (N=20) and response-toxicity outcomes

For control reasons, evaluation of gene expression of Interferon inducible genes (IFIGs, N=20) was performed using a bDNA multiplex assay (QGP 1.0, Panomics®). Only expression of Interferon stimulated exonuclease gene 20kDa (ISG20) differed significantly in all comparisons (P<0.035) before initiation of therapy with pegylated Interferon-α and ribavirin (PegIFN-α/RBV). Gene names and symbols listed in this table correspond to terms explained in EntrezGene: http://www.ncbi.nlm.nih.gov/gene

	overall ANOVA *P*-value	NR-NoPsAE vs. NR-PsAE	NR-NoPsAE vs. SVR-PsAE	NR-PsAE vs. SVR-PsAE
Gene Symbol	**P-value**	**P-value**	**P-value**	**P-value**
TRIM5	**9.64E-05**	0.618378	**5.35E-05**	**0.000242357**
APOBEC3G	**0.000130875**	0.416413	**5.37E-05**	**0.000526096**
OAS2	**0.00118548**	0.481903	**0.000462119**	**0.00316296**
IFIT1	**0.00125874**	0.268183	**0.000371204**	**0.00669527**
ISG20	**0.000180288**	**0.0343525**	**3.93E-05**	**0.0103719**
OAS1	**0.00708266**	0.579752	**0.00281471**	**0.0120099**
IFITM1	**0.00399497**	0.380528	**0.00129636**	**0.0122214**
IFIT3	0.0784446	0.497076	**0.0278317**	0.115344
MX1	0.0556843	0.335928	**0.0177016**	0.132397
MX2	0.0628115	0.341076	**0.0201968**	0.144085
STAT1	0.282406	0.930856	0.149474	0.183131
EIF2AK2	0.0879966	0.301756	**0.0290891**	0.216966
ISG15	0.0760249	0.260831	**0.0246772**	0.223032
IFI44	0.20454	0.428514	0.0779284	0.31044
PLSCR1	0.725129	0.812797	0.570623	0.438556
LY6E	0.208519	0.206418	0.0943669	0.640991
APOBEC3A	0.80747	0.532544	0.893426	0.644969
SP110	0.832107	0.841938	0.552333	0.694313
IFI27	0.803717	0.752103	0.513094	0.732396
IRF7	0.704648	0.412934	0.624917	0.763441

[SVR: sustained viral response; NR: viral non-response; PsAE: IFN-α induced psychiatric adverse effects; NoPsAE: No psychiatric adverse effects]

Table 2

Association between unique clinical outcomes (response or toxicity) and gene expression levels of ISG20, pre and post therapy

Association between unique clinical outcomes, i.e. viral response (SVR vs. NR) or neuropsychiatric toxicity (PsAE vs. NoPsAE), and gene expression of Interferon stimulated exonuclease gene 20kDa (ISG20) determined by microarray analysis and validated by bDNA assay at baseline (N=28 patients / Table 2A) and post therapy with pegylated Interferon-α and ribavirin (PegIFN-α/RBV) (N=25 patients / Table 2B).

A	Pre therapy (PegIFN-α/RBV)	ISG20 gene expression	P-value (ANOVA)
Response	**(microarray; HU133A/A_2, Affymetrix®)**	Up-regulated in NR	0.2016
Toxicity	**(microarray; HU133A/A_2, Affymetrix®)**	**Up-regulated in NoPsAE**	**0.0084**
Response	**(bDNA assay; QGP 1.0, Panomics®)**	**Up-regulated in NR**	**0.0003**
Toxicity	**(bDNA assay; QGP 1.0, Panomics®)**	**Up-regulated in NoPsAE**	**0.0010**

B	Post therapy (PegIFN-α/RBV)	ISG20 gene expression	P-value (ANOVA)
Response	**(microarray; HU133A/A_2, Affymetrix®)**	Up-regulated in NR	0.7604
Toxicity	**(microarray; HU133A/A_2, Affymetrix®)**	Up-regulated in PsAE	0.8975
Response	**(bDNA assay; QGP 1.0, Panomics®)**	Up-regulated in SVR	0.5887
Toxicity	**(bDNA assay; QGP 1.0, Panomics®)**	Up-regulated in NoPsAE	0.6180

[SVR: sustained viral response; NR: viral non-response; PsAE: IFN-α induced psychiatric adverse effects; NoPsAE: No psychiatric adverse effects]