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# Microarrays-Enabled Hypothesis Generation: The Suspect Role of FNBP-1 in Neuropsychiatric Pathogenesis Associated with HIV and/or HCV Infection

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## Abstract

**Objective—**The spectrum of neuropsychiatric illness (NI) associated with the Human Immunodeficiency Virus (HIV) and/or the Hepatitis C Virus (HCV) is far reaching and significantly impacts the clinical presentation and outcome of infected persons; however, the etiological and pathophysiological background remains partially understood. The present work was aimed to investigate the potential significance of formin binding protein 1 (FNBP-1)-dependent pathways in NI-pathogenesis by elaborating on previous microarray-based research in HIV and/or HCV-infected patients receiving interferon-α (IFN-α) immunotherapy via a rigorous data mining procedure.

**Methods—**Using microarray data of peripheral whole blood (PB) samples obtained from HCV mono-infected persons (n=25, Affymetrix® HG-U133A_2) 12 h before and after the 1st dose of pegylated IFN-α (PegIFN-α), we re-applied the same analytical algorithm that we had developed and published in an earlier study with HIV/HCV co-infected subjects (N=28, Affymetrix® HG-U133A), in order to evaluate reproducibility of potential NI-related molecular findings in an independent cohort.

**Results—**Among 28 gene expression profiles (HIV/HCV: N=9 vs. HCV: N=19) selected by applying different thresholds (a Mean Fold Difference value (MFD) in gene expression of ≥ 0.38 ($\log_2$) and/or P value from <0.05 to ≤ 0.1) FNBP-1 was identified as the only overlapping marker, which also exhibited a consistent upregulation in association with the development of NI in both cohorts. Previous functional annotation analysis had classified FNBP-1 as molecule with significant enrichment in various brain tissues (P<0.01).



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**Conclusion—**Our current findings are strongly arguing for intensifying research into the FNBP-1-related mechanisms that may be conferring risk for or resistance to HIV- and/or HCV-related NI.

### Keywords

Microarray; HIV; HCV; Neuropsychiatric disease; FNBP-1

## Introduction

Of 40 million people living worldwide with a Human Immunodeficiency Virus (HIV) infection approximately 2.3 million are chronically co-infected with the Hepatitis C Virus (HCV) [1]. HIV/HCV co-infected patients demonstrate higher rates of neuropsychiatric illness (NI, such as generalized anxiety disorder, dysthymia, panic disorder, major depression and substance abuse disorder) relative to HIV mono-infected subjects or the general population [2,3]. In previous research, we identified gene expression profiles significantly modulated in HIV/HCV co-infected patients who experienced pegylated interferon-α (PegIFN-α)-induced NI and were able to characterize the unique role of Interferon-stimulated-exonuclease-gene 20 kDa (ISG20) in linking PegIFN-α-related NI to distinct HCV treatment responses in patients co-infected with HIV and HCV [4,5]. Interestingly, during this work, we detected 9 molecular markers (i.e., chromosome 9 open reading frame 167; formin binding protein 1; spectrin repeat containing nuclear envelope 1; lanosterol synthase; IKAROS family zinc finger 1; single stranded DNA binding protein 3; arginyl aminopeptidase; ARP2 actin-related protein 2 homolog; Ubiquitin-conjugating enzyme E2I) that were characterized by sustained expression differences pre and post therapy between patients who developed PegIFN-α-related NI and those who did not [5]. These gene expression patterns along with functional annotation analysis results using the Database for Annotation, Visualization and Integrated Discovery (DAVID) [6] strongly suggest that this 9-gene signature likely contains HIV- and/or HCV-linked biology that renders the central nervous system (CNS) more vulnerable for NI, especially in the presence of systemic interferon [5,7–15]. Unfortunately, we were unable to validate these microarray data via real-time polymerase chain reaction (RT-PCR) as no peripheral blood mononuclear cell (PBMC) samples were available anymore from the HIV/HCV co-infected patients (N=28; 89% males; 50% African Americans; mean age: 46.5 years) enrolled in this study [5]. In an ultimate effort to evaluate reproducibility of these findings, we applied the same analytical and computational algorithms to peripheral whole blood (PB) samples collected from PegIFN-α naïve, HCV mono-infected patients (N=25; 64% males; 100% Caucasians; mean age: 43.6 years) who had been recruited for previous studies at the University Hospital in Essen (Essen, Germany). Despite the considerable heterogeneity of original study settings and demographics, a 2-step selection approach led to identification of formin binding protein 1 (FNBP-1) as a uniquely regulated marker in association with the development of NI in both cohorts. However, it has to be clear, that this analysis represents extension of previous work, which attempts to generate novel hypotheses underlying the molecular pathogenesis of HIV- and/or HCV-associated NI through a microarray data mining procedure. As modern standard HCV therapy consists exclusively of direct acting antivirals (DAA), these findings may still prove important to drive future investigation towards understanding the

pathogenesis of NI in patients requiring type I IFN therapies for diseases such as chronic hepatitis B virus infection [16,17], multiple sclerosis [17,18] or melanoma [17,19].

## Study Subjects and Methods

### Study subjects

25 therapy-naive patients with chronic HCV infection that were previously recruited at the University Hospital Essen (Essen, Germany) for microarray studies aimed to discover biomarkers for HCV-associated liver fibrosis and/or therapeutic response to subsequent PegIFN-α treatment (i.e., HCV elimination and drug-induced toxicity including NI) were considered for re-analysis for the purpose of this work. Inclusion and exclusion criteria, psychiatric evaluation and general study settings were applied as described elsewhere [20,21]. Methods of two earlier studies with PegIFN-α naive HIV/HCV co-infected subjects (N=28) recruited at the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH, Bethesda, Maryland, USA), which led to identification of FNBP-1 and provided foundation for this research, are published elsewhere [4,5]. Overall, study subjects signed informed consents approved by the institutional review board prior to enrollment.

### Microarray analysis

PB samples collected from HCV mono-infected patients (N=25) were subjected to microarray analysis (Affymetrix® HG-U133A_2) as previously described [4,5,20–22].

### First step

One-way-ANOVA (PARTEK Genomics Suite) was performed based on assigning HCV mono-infected patients (N=25) patients into two groups, i.e. those who developed NI and those who did not (NoNI); a Mean Fold Difference value (MFD) has been calculated for each selected gene before the first administration of PegIFN-α representing the absolute mean difference in gene expression ($\log_2$) between the groups. Since statistical comparisons between gene expression signals measured in different specimens (PB vs. PMBC) was intended, we applied an absolute MFD filter of ≥ 0.38 ($\log_2$) but relaxed the P value threshold from <0.05 to ≤ 0.1 in order to increase power of bioinformatics analysis performance at baseline. Because induced expression signals were measured for each gene 12 h after the 1st administration of PegIFN-α in HCV mono-infected patients (and not at the end of a 48 weeks treatment, as had been the case in HIV/HCV co-infected study participants) we set a MFD threshold of ≥ 0.38 ($\log_2$) as the only gene selection filter for analysis at this time point. Hence, four subsets (A, B, C, D) were formed including genes that passed these criteria (Table 1).

### Second step

Venn Diagrams were performed based on these four subsets with the intention to overlap significant results from the group comparison NI vs. NoNI pre and/or post treatment to identify common gene signatures between both cohorts (Figure 1). Furthermore, two sets (E and F) including nine and nineteen unique genes, respectively, were found to have passed the selection cutoffs in the abovementioned contrasts.

## Results

Using a combined statistical algorithm as described under METHODS, two subsets (E and F) representing a total of 28 unique genes were selected. Among those, only FNBP-1 was characterized by homologous expression patterns, i.e., upregulation, in association with NI in both cohorts, especially at baseline, which represents the best comparable condition between both studies, but also during PegIFN-α based treatment (Figure 1 and Table 2). Moreover, FNBP-1 counts to molecular markers that demonstrate significant enrichment in various brain tissues ($P<0.01$) according to functional annotation tool “DAVID” [5,6].

The statistical variability witnessed between both cohorts may be attributed by differences in blood samples, RNA quality, racial type, and the co-incidence of HIV infection; the latter factor may influence differential regulation with respect to NI pathogenesis more drastically than HCV mono-infection [23].

Interestingly, correlation of FNBP-1 gene expression values between two registered probesets, i.e., the 212288_at and the 213940_s_at, was relatively poor (HCV: $r=0.29$/ $P=0.145$) or even inverse (HIV/HCV: $r=-0.34$/$P=0.07$) at baseline and post treatment in the HIV/HCV cohort ($r=0.31$/$P=0.119$, Table 3), likely due to expression of multiple transcript variants. Probesets, which are designated by the suffix “s_at”, detect transcript variants sharing common sequence (http://www.affymetrix.com/support/help/faqs/hgu133/faq_2.jsp). Such splicing variants of FNBP-1 with functionally different responses in the CNS have been previously reported [24].

## Discussion

Microarray analysis revealed that upregulation of FNBP-1 gene expression at baseline appears as a marker of a clinical state characterized by increased vulnerability for subsequent NI in association with HIV/HCV co-infection and to lesser degree to HCV mono-infection. Interestingly, in human samples, FNBP-1 has been found to be recruited to clathrin-coated pits in clathrin-mediated endocytosis (CME), which suggests the physiological role of FNBP-1 in this ubiquitous process [25]. In line with this observation, FNBP-1 is also reportedly involved in dynamin-mediated endocytosis in a clathrin-dependent as well as clathrin-independent manner [26,27]. Importantly, inhibition of clathrin-dependent endocytosis has been shown to block uptake of IFN-α receptors resulting in attenuation of IFN-α-induced signaling, and thus, likely also of NI pathogenesis driven by IFN-α [28]. In agreement with these data, we found a consistently significant positive correlation between gene expression values of FNBP-1 and clathrin, heavy chain (CLTC; 200614_at) in the HIV/HCV co-infected ($P_{pre}<0.043$) as well as the HCV mono-infected cohort ($P_{pre}<0.01$); notably, CLTC gene depletion has been characterized as an effective method of inhibiting CME in cell lines [29]. The consistent correlation dynamics between FNBP-1, CLTC and NI in two independent cohorts supports the biological validity of this result.

Furthermore, it has been previously reported that symptoms of behavioral illness can be reliably reproduced in animals and humans by administration of cytokines such as

interleukin-1 (IL-1), interleukin-6 (IL-6) and Tumor Necrosis Factor alpha (TNFα) [30–32]. Against this background, studies suggesting involvement of clathrin-mediated mechanisms in IL-1 internalization [33], intracellular trafficking and transcytosis of TNFα [34], as well as endocytosis of the IL-6 receptor complex [35] along with induction of pro-inflammatory cytokines, including IL-1, IL-6 and TNFα by PegIFN-α [36,37], imply a multifaceted role of FNBP-1 in the pathogenesis of NI.

Moreover, a potential role of FNBP-1 with regard to NI may further derive from studies revealing the significant influence of serotonin on the activity of excitatory synapses in prefrontal cortex pyramidal neurons. In fact, serotonin regulates synaptic plasticity through a mechanism facilitating clathrin/dynamin-dependent internalization of ionotropic glutamate (AMPA) receptors [38]. The possibility that AMPA receptor trafficking also may be involved in the pathophysiology of neuropsychiatric disorders is suggested by recent studies showing the ability of abuse substances to elevate levels of the AMPA receptors in the ventral tegmental area as crucial for the development of behavioral sensitization [39]. Recently, FNBP-1 was reported to interact with sorting nexin 2 (SNX2), a molecular factor involved in several stages of intracellular trafficking [40], suggesting that FNBP-1 is also affecting receptor trafficking. Additionally, CME represents the primer mechanism of vesicle retrieval in hippocampal synapses [29], which indicates a further pathway of how FNBP-1 might be implicated in the regulation of CNS functions potentially relevant for the pathogenesis of psychiatric disorders.

Taken together, these data demonstrate that FNBP-1 plays a potential role in mediating immune-related neuropsychiatric illness. Moreover, the reported influence of FNBP-1 on monoamine pathways, which are considered of crucial importance in the pathogenesis and treatment of neuropsychiatric disorders [41], suggests further major involvement of this molecule in regulation of neuropsychological functions in the CNS.

Although not validated, which represents the major limitation of this work, here, we presented microarray data strongly arguing for intensifying research into the FNBP-1-related mechanisms that may be conferring risk for/resistance to interferon-induced neuropsychiatric events. Obviously, without striving for samples homology or including further control groups (i.e., HBV mono-, HBV/HIV co-infected, non-infected patients with IFN-α therapy and non-infected persons with primary psychiatric disorders, etc.) in proper study designs and without performing experimental/functional validation of microarray analysis results, it will be difficult to reach for evident mechanisms causing neuropsychiatric illness beyond the descriptive and/or hypothetical interpretation of these data.

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## References


1. Platt L, Easterbrook P, Gower E, McDonald B, Sabin K, et al. Prevalence and burden of HCV co-infection in people living with HIV: A global systematic review and meta-analysis. Lancet Infect Dis. 2016; 16:797–808. [PubMed: 26922272]
2. Weiss JJ, Gorman JM. Psychiatric behavioral aspects of comanagement of hepatitis C virus and HIV. Curr HIV/AIDS Rep. 2006; 3:176–181. [PubMed: 17032577]
3. Goulet JL, Fultz SL, McGinnis KA, Justice AC. Relative prevalence of comorbidities and treatment contraindications in HIV-mono-infected and HIV/HCV-co-infected veterans. AIDS. 2005; 19:S99–S105.
4. Rasimas J, Katsounas A, Raza H, Murphy AA, Yang J, et al. Gene expression profiles predict emergence of psychiatric adverse events in HIV/HCV-coinfected patients on interferon-based HCV therapy. J Acquir Immune Defic Syndr. 2012; 60:273–281. [PubMed: 22728749]
5. Katsounas A, Rasimas JJ, Schlaak JF, Lempicki RA, Rosenstein DL, et al. Interferon stimulated exonuclease gene 20 kDa links psychiatric events to distinct hepatitis C virus responses in human immunodeficiency virus positive patients. J Med Virol. 2014; 86:1323–1331. [PubMed: 24782267]
6. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009; 4:44–57. [PubMed: 19131956]
7. Simmen FA. The Kruppel-like factor 9 (KLF9) network in HEC-1-A endometrial carcinoma cells suggests the carcinogenic potential of dysregulated KLF9 expression. Reprod Biol Endocrinol. 2008; 6:41. [PubMed: 18783612]
8. Zhang J, Moseley A, Jegga AG, Gupta A, Witte DP, et al. Neural system-enriched gene expression: Relationship to biological pathways and neurological diseases. Physiol Genomics. 2004; 18:167–183. [PubMed: 15126645]
9. Le-Niculescu H, Amy M, Jegga AG, Gupta A, Witte DP, et al. Convergent functional genomics of genome-wide association data for bipolar disorder: Comprehensive identification of candidate genes, pathways and mechanisms. Am J Med Genet B Neuropsychiatr Genet. 2009; 150:155–181.
10. Beasley CL, Honer WG, Bergmann K, Falkai P, Lütjohann D, et al. Reductions in cholesterol and synaptic markers in association cortex in mood disorders. Bipolar Disord. 2005; 7:449–455. [PubMed: 16176438]
11. Han L, Wong DL, Tsai G, Jiang Z, Coyle JT. Promoter analysis of human glutamate carboxypeptidase II. Brain Res. 2007; 1170:1–12. [PubMed: 17689503]
12. Wu L. Structure and functional characterization of single-strand DNA binding protein SSDP1: Carboxyl-terminal of SSDP1 has transcription activity. Biochem Biophys Res Commun. 2006; 339:977–984. [PubMed: 16325762]
13. Cicin-Sain L, Simaga S, Froebe A, Abramić M. Central aminopeptidase and serotonin system activities: Possible relationship. Neuropeptides. 2008; 42:435–440. [PubMed: 18547641]
14. Wegner AM, Nebhan CA, Hu L, Majumdar D, Meier KM, et al. N-wasp and the arp2/3 complex are critical regulators of actin in the development of dendritic spines and synapses. J Biol Chem. 2008; 283:15912–15920. [PubMed: 18430734]
15. Jentsch S. The ubiquitin-conjugation system. Annu Rev Genet. 1992; 26:179–207. [PubMed: 1336336]
16. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, et al. Peginterferon Alfa-2a, lamivudine and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005; 352:2682–2695. [PubMed: 15987917]
17. Zdilar D, Franco-Bronson K, Buchler N, Locala JA, Younossi ZM. Hepatitis C, interferon alfa, and depression. Hepatology. 2000; 31:1207–1211. [PubMed: 10827143]
18. Rieckmann P, Toyka KV, Bassetti C, Beer K, Beer S, et al. Escalating immunotherapy of multiple sclerosis--new aspects and practical application. J Neurol. 2004; 251:1329–1339. [PubMed: 15592728]
19. Mocellin S, Pasquali S, Rossi CR, Nitti D. Interferon alpha adjuvant therapy in patients with high-risk melanoma: A systematic review and meta-analysis. J Natl Cancer Inst. 2010; 102:493–501. [PubMed: 20179267]

20. Schlaak JF, Trippler M, Hoyo-Becerra C, Erim Y, Kis B, et al. Selective hyper-responsiveness of the interferon system in major depressive disorders and depression induced by interferon therapy. PLoS One. 2012; 7:e38668. [PubMed: 22701688]
21. Katsounas A, Trippler M, Wang B, Polis M, Lempicki RA, et al. CCL5 mRNA is a marker for early fibrosis in chronic hepatitis C and is regulated by interferon-α therapy and toll-like receptor 3 signalling. J Viral Hepat. 2012; 19:128–137. [PubMed: 22239502]
22. Lempicki RA, Polis MA, Yang J, McLaughlin M, Koratich C, et al. Gene expression profiles in hepatitis C virus (HCV) and HIV coinfection: class prediction analyses before treatment predict the outcome of anti-HCV therapy among HIV-coinfected persons. J Infect Dis. 2006; 193:1172–1177. [PubMed: 16544259]
23. Forton DM, Allsop JM, Cox IJ, Hamilton G, Wesnes K, et al. A review of cognitive impairment and cerebral metabolite abnormalities in patients with hepatitis C infection. AIDS. 2005; 19:S53–S63.
24. Kakimoto T, Katoh H, Negishi M. Identification of splicing variants of Rapostlin, a novel RND2 effector that interacts with neural Wiskott-Aldrich syndrome protein and induces neurite branching. J Biol Chem. 2004; 279:14104–14110. [PubMed: 14732713]
25. Shimada A, Niwa H, Tsujita K, Suetsugu S, Nitta K, et al. Curved EFC/F-BAR-domain dimers are joined end to end into a filament for membrane invagination in endocytosis. Cell. 2007; 129:761–772. [PubMed: 17512409]
26. Fujita H, Katoh H, Ishikawa Y, Mori K, Negishi M. Rapostlin is a novel effector of Rnd2 GTPase inducing neurite branching. J Biol Chem. 2002; 277:45428–45434. [PubMed: 12244061]
27. Kamioka Y, Fukuhara S, Sawa H, Nagashima K, Masuda M, et al. A novel dynamin-associating molecule, formin-binding protein 17, induces tubular membrane invaginations and participates in endocytosis. J Biol Chem. 2004; 279:40091–40099. [PubMed: 15252009]
28. Marchetti M, Monier MN, Fradagrada A, Mitchell K, Baychelier F, et al. Stat-mediated signaling induced by type I and type II interferons (IFNs) is differentially controlled through lipid microdomain association and clathrin-dependent endocytosis of IFN receptors. Mol Biol Cell. 2006; 17:2896–2909. [PubMed: 16624862]
29. Granseth B, Odermatt B, Royle SJ, Lagnado L. Clathrin-mediated endocytosis is the dominant mechanism of vesicle retrieval at hippocampal synapses. Neuron. 2006; 51:773–786. [PubMed: 16982422]
30. Capuron L, Miller AH. Cytokines and psychopathology: Lessons from interferon-alpha. Biol Psychiatry. 2004; 56:819–824. [PubMed: 15576057]
31. Reichenberg A, Kraus T, Haack M, Schuld A, Pollmächer T, et al. Endotoxin-induced changes in food consumption in healthy volunteers are associated with TNF-alpha and IL-6 secretion. Psychoneuroendocrinology. 2002; 27:945–956. [PubMed: 12383455]
32. Spath-Schwalbe E, Hansen K, Schmidt F, Schrezenmeier H, Marshall L, et al. Acute effects of recombinant human interleukin-6 on endocrine and central nervous sleep functions in healthy men. J Clin Endocrinol Metab. 1998; 83:1573–1579. [PubMed: 9589658]
33. Bourke E, Cassetti A, Villa A, Fadlon E, Colotta F, et al. IL-1 beta scavenging by the type II IL-1 decoy receptor in human neutrophils. J Immunol. 2003; 170:5999–6005. [PubMed: 12794127]
34. Pan W, Kastin AJ. Tumor necrosis factor and stroke: Role of the blood-brain barrier. Prog Neurobiol. 2007; 83:363–374. [PubMed: 17913328]
35. Thiel S, Dahmen H, Martens A, Müller-Newen G, Schaper F, et al. Constitutive internalization and association with adaptor protein-2 of the interleukin-6 signal transducer gp130. FEBS Lett. 1998; 441:231–234. [PubMed: 9883890]
36. Taylor JL, Grossberg SE. The effects of interferon-alpha on the production and action of other cytokines. Semin Oncol. 1998; 25:23–29.
37. Capuron L, Raison CL, Musselman DL, Lawson DH, Nemeroff CB, et al. Association of exaggerated HPA axis response to the initial injection of interferon-alpha with development of depression during interferon-alpha therapy. Am J Psychiatry. 2003; 160:1342–1345. [PubMed: 12832253]

38. Zhong P, Liu W, Gu Z, Yan Z. Serotonin facilitates long-term depression induction in prefrontal cortex via p38 MAPK/Rab5-mediated enhancement of AMPA receptor internalization. J Physiol. 2008; 586:4465–4479. [PubMed: 18653660]

39. Carlezon WA Jr, Nestler EJ. Elevated levels of GluR1 in the midbrain: A trigger for sensitization to drugs of abuse? Trends Neurosci. 2002; 25:610–615. [PubMed: 12446127]

40. Fuchs U, Rehkamp G, Haas OA, Slany R, Kōnig M, et al. The human formin-binding protein 17 (FBP17) interacts with sorting nexin, SNX2 and is an MLL-fusion partner in acute myelogeneous leukemia. Proc Natl Acad Sci USA. 2001; 98:8756–8761. [PubMed: 11438682]

41. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med. 2001; 344:961–966. [PubMed: 11274622]

## Abbreviations

| | |
|---|---|
| **HIV/HCV** | Co-infection with the Human Immunodeficiency Virus (HIV) and the Hepatitis C Virus (HCV) |
| **HCV** | Mono-infection with the Hepatitis C Virus (HCV) |
| **NI** | IFN-α-Induced Neuropsychiatric Illness |
| **NoNI** | No Neuropsychiatric Illness |
| **Pre** | Before Administration of PegIFN-α Based Therapy |
| **Post** | After Administration of PegIFN-α Therapy (for HIV/HCV co-infected patients: 48 weeks unless discontinued earlier for adverse events for HCV Mono-Infected Patients: 12 h after Administration of the 1$^{st}$ PegIFN-α injection) |
| **MFD** | Mean Fold Difference |
| **UHE** | University Hospital Essen |
| **NIAID** | National Institute of Allergy and Infectious Diseases |
| **NIH** | National Institutes of Health |
| **PBMC** | Peripheral Blood Mononuclear Cell |
| **PB** | Peripheral Whole Blood |
| **FNBP-1** | Formin Binding Protein 1 |
| **Partial Corr** | Partial Correlation |
| **NI>NoNI** | FNBP-1 Gene Expression was Increased in Patients who Developed NI Relative to those who did not (NoNI) |

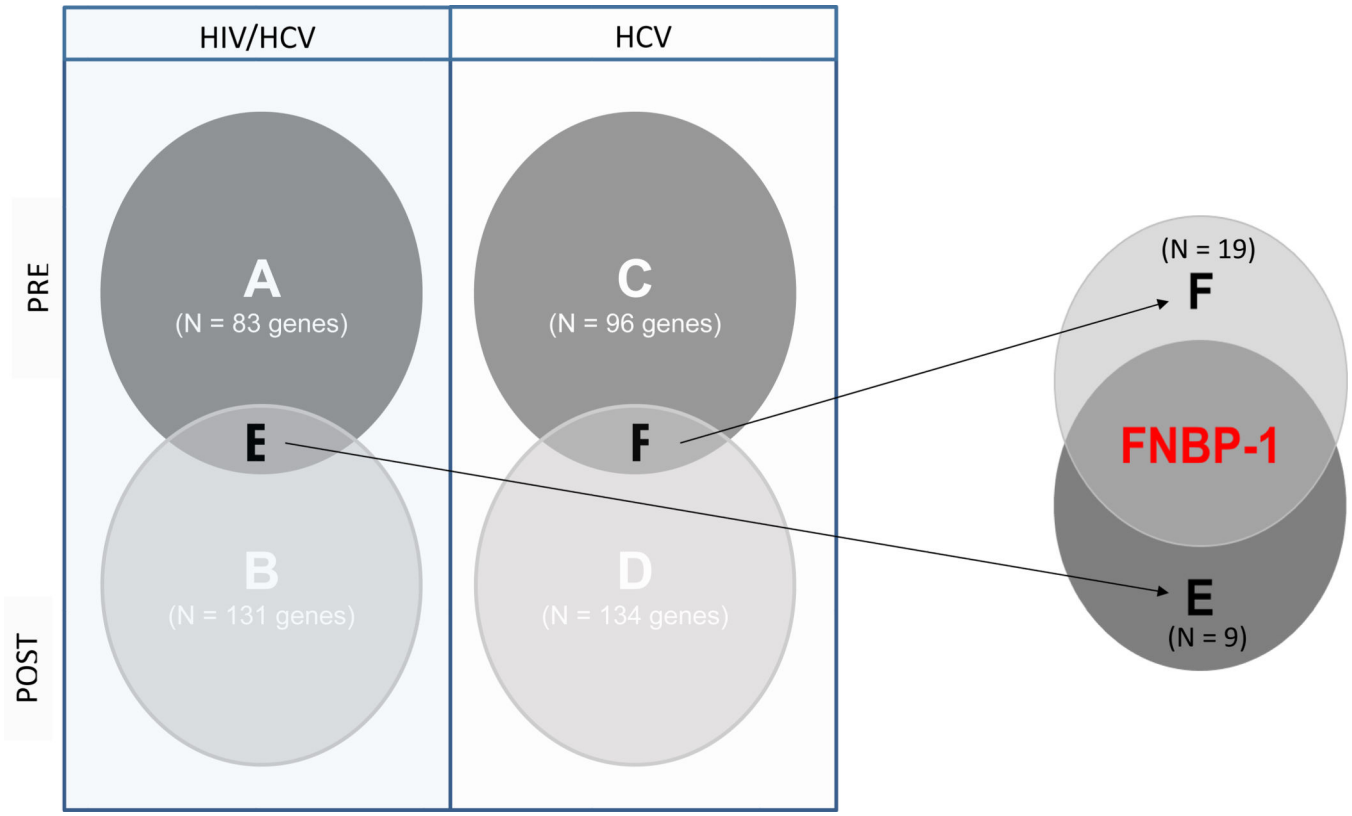


**Figure 1.**
Venn diagrams.

**Table 1**

Infection status, patient groups, statistical criteria and gene subsets along with gene counts per selected subset.

| Infection (patients) | Time | Study | Contrast | Selection criteria | Genes (N) | Gene subset |
|---|---|---|---|---|---|---|
| **HIV/HCV (N=28)** | Pre | NIAID/NIH (MD, USA) | NoNI-NI | P<0.05 and MFD (log2) ≥ 0.38 | (83) | A |
| **HIV/HCV (N=25)** | Post | NIAID/NIH (MD, USA) | NoNI-NI | P<0.05 and MFD (log2) ≥ 0.38 | (131) | B |
| **HCV (N=25)** | Pre | UHE (Germany) | NoNI-NI | P≤0.1 and MFD (log2) ≥ 0.38 | (96) | C |
| **HCV (N=25)** | Post | UHE (Germany) | NoNI-NI | MFD (log2) ≥ 0.38 | (134) | D |

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**Table 2**

Comparison of FNBP-1 gene expression levels between both cohorts.

| FNBP-1 212288_at | HCV (PB) HG-U133A_2 | P value | HIV/HCV (PBMC) HG-U133A | P value |
|---|---|---|---|---|
| **Pre** | NI>NoNI | 0.101 | NI>NoNI | 0.006 |
| **Post** | NI>NoNI | 0.385 | NI>NoNI | 0.048 |

**Table 3**

Linear regression analysis between FNBP-1 gene expression levels measured via different probesets: 213940_s_at versus 212288_at.

| FNBP-1 | Probeset | HCV (PB) HU133A_2 | | HIV/HCV (PBMC) HU133A | |
|---|---|---|---|---|---|
| | | Partial Corr. | P value | Partial Corr. | P value |
| | | 212288_at | | | |
| Pre | 213940_s_at | 0.29 | 0.145 | −0.34 | 0.070 |
| Post | | (12 h) 0.63 | (12 h) 0.0006 | (48 weeks) 0.31 | (48 weeks) 0.119 |