Identification of the transcripts associated with spontaneous HCV clearance in individuals co-infected with HIV and HCV

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Abstract

Background: Infection with human immunodeficiency virus (HIV) influences the outcome and natural disease progression of hepatitis C virus (HCV) infection. While the majority of HCV mono-infected and HCV/HIV co-infected subjects develop chronic HCV infection, 20–46% of mono- and co-infected subjects spontaneously clear HCV infection. The mechanism underlying viral clearance is not clearly understood. Analysis of differential cellular gene expression (mRNA) between HIV-infected patients with persistent HCV infection or spontaneous clearance could provide a unique opportunity to decipher the mechanism of HCV clearance.

Methods: Plasma RNA from HIV/HCV co-infected subjects who cleared HCV and those who remained chronically infected with HCV was sequenced using Ion Torrent technology. The sequencing results were analyzed to identify transcripts that are associated with HCV clearance by measuring differential gene expression in HIV/HCV co-infected subjects who cleared HCV and those who remained chronically infected with HCV.

Results: We have identified plasma mRNA, the levels of which are significantly elevated (at least 5 fold, False Discovery Rate (FDR) <0.05) before HCV infection in subjects who cleared HCV compared to those who remained chronically infected. Upon further analysis of these differentially expressed genes, before and after HCV infection, we found that before HCV infection 12 genes were uniquely upregulated in the clearance group compared to the chronically infected group. Importantly, a number of these 12 genes and their upstream regulators (such as CCL3, IL17D, LBP, SOCS3, NFKBIL1, IRF) are associated with innate immune response functions.

Conclusions: These results suggest that subjects who spontaneously clear HCV may express these unique genes associated with innate immune functions.

Keywords: HIV/HCV coinfection, Clearance of HCV infection, Chronic HCV infection, Plasma RNA sequencing, Sequence analysis
reported to be associated with HCV clearance. Various factors, such as gene polymorphisms in interleukin 28B (IL28B) and IP-10 have been implicated in mediating host susceptibility/resistance to HCV infection and disease progression [14–17]. In HCV-infected chimpanzees, interleukin binding factor 3 (ILF3) and cytotoxic granule associated cRNA binding protein (TIA1), which are associated with robust T-cell responses, were highly induced in animals who cleared the virus [18]. However, these represent only a few of the many factors that are potentially involved in HCV clearance.

Differential cellular gene expression in HIV-infected subjects has been shown to be associated with HIV disease progression [19]. Therefore, analysis of differential cellular gene expression between HIV infected patients with persistent HCV infection and those with spontaneous clearance of this virus could provide a unique opportunity to decipher the mechanism of HCV clearance in HIV/HCV co-infected subjects.

Pathogenic changes occurring in any tissues or organs are expected to leave footprints in the blood. Consequently, measurement of certain target RNA in the plasma has been explored or used for diagnosis of a number of diseases and cancers [20–24]. We hypothesize that the cellular (immunological and non-immunological) factors that are responsible for spontaneous HCV clearance leave footprints in the plasma, leading to differential plasma RNA expression profiles between those who spontaneously clear HCV infection and chronically infected individuals, with or without HIV co-infection. It is presumed that differential gene expression in plasma between those who cleared HCV and those who remained chronically infected will provide clues to the mechanism of HCV clearance. Due to the low quantity of RNA in blood plasma, currently there is no convenient way to characterize plasma RNA profiles. Recent advancement of next-generation sequencing technologies has made it possible for unbiased and comprehensive analysis of the gene expression from both cells and tissues. We have recently modified the NGS technology in Ion Proton platform and characterized RNA profiles in plasma.

In this study we identified plasma mRNA, the levels of which are significantly increased (at least 5 fold, FDR <0.05) in the HCV clearance group compared to chronically HCV infected patients. Upon further analysis of these differentially expressed genes, we have identified 12 genes that are upregulated only in clearance group before HCV infection. Moreover, some of these 12 genes and their upstream regulators are associated with innate immune functions.

Methods
Patients and samples
Frozen plasma samples were obtained from 13 HCV seroconverters who were infected with HIV for more than 10 years before HCV seroconversion in the Multicenter AIDS Cohort Study (MACS) [25]. MACS is the first and largest study specifically created to examine the natural history of AIDS. MACS participants are seen every six months and at each visit, plasma is taken for storage at -80 °C. HCV seroconversion is defined by the HCV antibody switching from negative to positive in plasma and the plasma HCV antibody positivity were persistent for all subsequent visits of the individuals. Furthermore, HCV seroconversion visits were confirmed by reverse transcriptase polymerase chain reaction specific for detecting HCV RNA. Spontaneous HCV clearance is defined as plasma HCV RNA were never detected or detected only one to two times around the time of seroconversion. Chronic HCV infection was defined as plasma HCV RNA being persistently detected for more than five years. From five subjects with spontaneous HCV clearance and eight subjects with chronic HCV infection, we examined their plasma samples that were obtained immediately before and after HCV infection (MACS visits are six months apart). All HCV infections were reported to have been acquired by sexual transmission. At the time of HCV infection, all subjects were naive to antiretroviral therapy and anti-HCV treatment.

Nucleic acid extraction, cDNA library construction and sequencing
Total nucleic acids were extracted from 1 ml of frozen cryopreserved plasma using an automated NucliSens EasyMag nucleic acid extraction machine (bioMérieux, Durham, NC) followed by removal of DNA from the nucleic extract with Qiaen AllPrep DNA/RNA mini kit and ribosomal RNA by a Low Input RiboMinus System (Life Technologies). The cDNA Library was then constructed using an Ion Torrent Total RNA-Seq Kit (Life Technologies) for whole transcriptome libraries and Barcodes 1 through 8 from an Ion Xpress 1–16 barcoding kit were used (Life Technologies) for each individual sample. cDNA libraries were quantified by qPCR using an Ion Library Quantitation Kit (Life Technologies) to determine a suitable template dilution factor for subsequent emulsion PCR and sequencing.

Four barcoded samples were combined for one sequencing reaction. Template preparation for sequencing was conducted using the OneTouch Ion™ Template Kit in the OneTouch machine (Life Technologies). Ion Torrent sequencing was conducted using the Ion Proton Sequencing Kit (Life Technologies) on an Ion Proton Machine (Life Technologies) using a P1(v2)-chip (Life Technologies). The constructed cDNA was also used for HIV viral load measurement using a quantitative real time PCR for HIV gag RNA, with a sensitivity of 10 copies/mL [26].
**Sequence analysis**

Raw sequencing reads in FastQ format were assessed for quality using CLC Genomics Workbench 7. Reads were accepted based on the length (longer than 25 nucleotides) and number of ambiguous bases (Phred Quality Score higher than 20). Quality trimming was performed. The mean numbers of reads from different groups after trimming ranged from 3.2 million to 8.4 million. The trimmed reads were then mapped to Homo sapiens gene sequences based on Homo sapiens (hg19) mRNA.

Bioconductor edgeR in R package was employed to perform the differential expression analysis. A general linear model was applied on the subjects before and after HCV infection to accommodate the multifactor design of the experiment. The model incorporates the main effect for HCV infection plus interactions with patients and viral clearance, thus allowing us to identify genes differentially expressed in HCV cleared and chronically infected patients before and after HCV infection. To ensure there were sufficient counts for each gene in the test, genes with mean read counts higher than 10 were kept in the analysis. Genes with Benjamini-Hochberg adjusted FDR <0.05 and fold change greater than 1 were considered as significant genes. The significance and function networks of the detected differentially expressed genes were analyzed using Ingenuity Pathway Analysis software. The gene function information was obtained from GeneCards Human Gene Database (www.genecards.org).

**Results**

**Characteristics of study participants**

A total of 26 plasma samples from the 13 HCV seroconverters obtained within six months before and after HCV infection were analyzed for plasma transcriptome profiles. All the participants were HIV positive for more than 10 years before HCV infection occurred. Four of the five participants who spontaneously cleared HCV infection had the CC variant of the IL28B gene, while the remaining one had the TT variant. In contrast, half of eight participants who became chronically infected by HCV had the CC variant IL28B gene and the remaining participants had the CT variant. For the 5 individuals with spontaneous HCV clearance, HCV loads were undetectable at the first HCV antibody positive visit. In contrast, the eight participants with chronic HCV infection had a median plasma HCV load of 1.6x10^7 copies/ml at the first HCV RNA positive visit, with values ranging from 1.22 x 10^6 to 7 x 10^7 (Table 1). In the HCV clearance group, the HIV viral load was below 40 copies/ml in three of the five patients and >71,000 copies/ml in two participants both before and after HCV seroconversion. In the chronically HCV infected group, HIV viral load ranged from 360 to 465,000 copies/ml (mean 125,233 copies/ml) (Table 1).

**Plasma mRNA profile of HIV-infected individuals with HCV clearance and those with chronic infection**

Plasma transcriptome analysis was performed in HCV infected individuals who cleared virus and those who remained chronically infected. A schematic diagram of the approaches for measurement of differentially expressed genes from the patients’ plasma samples collected before and after HCV infection and those cleared HCV and those who remained chronically infected is shown in Fig. 1. A comparison of transcriptomes before HCV infection...
between the HCV clearance group (TR1B, Fig. 1) and the chronically HCV infected group (TR2B, Fig. 1) showed that the expression levels of 32 genes were significantly higher (5–563 fold) and expression level of one gene (LL22NC03–63E9.3) was significantly lower in the clearance group (Table 2). To further assess the clustering of subjects within the chronic and clearance groups and across the groups, hierarchical clustering was performed using the differentially expressed genes (Fig. 2a). Four of the eight chronically infected participants clustered together indicating a distinct gene expression pattern whereas the other four samples did not show a similar pattern. On the other hand, three of the five participants in the clearance group clustered indicating similar gene expression and the remaining two subjects had different gene expression. In both clearance and chronic groups, the characteristics of the samples that did not aggregate with the rest of the samples in the respective groups were not different with respect to HIV viral load or IL28B polymorphism. This suggests that HIV viral load and IL28B polymorphism may not have an independent effect on the pattern of the gene expression.

Of the 33 genes that were differentially expressed (DEGs) before HCV infection, 19 DEGs have known biological functions (Gene Cards Human Gene Database (http://www.genecards.org/)): six gene products (18%) (ATP6V1G2, LPAR1, GNAI1, CCL3, IL17D, SLC2A6) are related to signal transduction pathways and innate immune response, eight gene products (24%) (LBR, EARS2, RAB11FIP5, CHST10, PRPSAP1, PCMTD2, MCU, PRDM13) are involved in metabolism and protein trafficking, and five gene products (15%) (ZFHX3, DAZA, TLX3, HOXD13, NFKBIL1) are RNA/DNA binding proteins involved in gene regulation (Fig. 2b).

To determine the differential gene expression pattern in response to HCV infection, a similar analysis was performed between transcriptomes in the clearance group (TR1A, Fig. 1) and chronic infection group (TR2A, Fig. 1), after HCV infection (Fig. 1). The expression levels of 56 genes were significantly higher, and that of one gene was significantly lower, in TR1A compared to TR2A (Table 3). Hierarchical clustering of DEGs showed that genes from 5 patients who had chronic infection clustered together, while no such clustering pattern was observed in the clearance group (Fig. 3a).

Of the 56 DEGs in the HCV clearance group, 34 are known to encode biologically functional proteins. Of these 34 genes, 14 DEG gene products (25%) (MIXL1, SLC30A3, SFRP5, NLRP13, IRS4, GNAI1, CMTM4, F10, LPAR1, ATP12A, TLX3, ATP6V1G2, NEURL, AP3B1) are known to participate in, or to be related to, signal transduction pathways and innate immune responses, twelve DEG gene products (21%) (LBR, MTMR1, PPP1R3G, PRPSAP1, RAB11FIP5, MANEAL, MCU, ADPRHL1, PRDM13, DPY19L3, SLC51A, CHST10) are involved in metabolism and protein trafficking and eight DEG gene products (14%) (ZFHX3, SOHLH1, TCF15, ZC3H8, ZXD8, TLX3, HOXD13, ZRXA) are RNA/DNA binding proteins involved in gene regulation (Fig. 3b). However, no DEG was identified between plasma samples analyzed before and after HCV infection, regardless whether they were from clearance group (TR1B vs. TR1A) or chronic group (TR2B vs. TR2A) (Fig. 1).

Intersection analyses between DEGs identified in the clearance group before HCV infection (DEG-B, Fig. 1) and after HCV infection (DEG-A, Fig. 1) indicate that 12 DEGs were uniquely expressed in clearance group before HCV infection and 36 DEGs were uniquely expressed after HCV infection (Fig. 4). There were 21 DEGs that were common between the two groups. IPA analyses of these unique 12 genes expressed before HCV infection indicate that a number of these genes and their upstream regulators are associated with innate immune response in the clearance group (Fig. 5a and b). Among them 14% are involved in immune cell trafficking, 14% are involved in humoral immune response and 14% are involved in cell mediated immune response suggesting that most of the unique DEGs in the clearance group before infection may play a role in defending against the virus (Fig. 5a). For instance, cytokine (such as IL-1, IL-4, IL-6, IL-8, IL-9, IL-10, TNFR1, interferon) signaling, Toll like receptor (TLR) signaling, MAPK signaling, NF-kB signaling, and communication between innate and adaptive immune cells may be involved (Fig. 5b). Similar analyses with 36 DEGs after HCV infection found that these genes are involved in glucocorticoid receptor signaling, myc mediated apoptosis signaling, axonal guidance signaling, IGF1 signaling, MAPK
### Table 2 DEGs in plasma before HCV infection in clearance group compared to those in chronically infected group

| DEGs* | Gene function                                                                 | Fold difference | P value   | FDR      |
|-------|--------------------------------------------------------------------------------|-----------------|-----------|----------|
| AD000091.2 | Uncharacterized                                                                 | 563.4           | 2.83E-07 | 0.001188912 |
| C11orf95 | Chromosome 11 open reading frame 95. Diseases associated with C11orf95 include ependymoma and chondroid lipoma. | 100.9           | 5.80E-07 | 0.001188912 |
| LPAR1 | Lysophosphatidic acid receptor 1. Its related pathways are PI3K-Akt signaling pathway and Ras signaling pathway. | 20              | 6.66E-07 | 0.001188912 |
| LBR | Lamin B receptor. Its related pathways are Metabolism and Metabolism. | 20.6            | 2.85E-06 | 0.003815476 |
| GNAI1 | Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1. Its related pathways are GPCR Pathway and GPCR Pathway. | 7.6             | 9.21E-06 | 0.008563212 |
| HOXD13 | Homeobox D13. This gene is related to sequence-specific DNA binding transcription factor activity and chromatin binding. | 231.4           | 9.61E-06 | 0.008563212 |
| EARS2 | Glutamyl-tRNA synthetase 2, mitochondrial. Its related pathways are Metabolism and Gene Expression. | 20              | 6.66E-07 | 0.008563212 |
| PRDM13 | PR domain containing 13. This gene is related to methyltransferase activity. | 30.4            | 1.28E-05 | 0.008563212 |
| RAB11FIP5 | RAB11 family interacting protein 5 (class I). Its related pathways are Endocytosis and Delta508-CFTR traffic/ER-to-Golgi in CF. | 23              | 2.52E-05 | 0.013834245 |
| CTC-504A5.1 | Affiliated with the ncRNA class. | 16.6            | 2.58E-05 | 0.013834245 |
| CHST10 | Carbohydrate sulfotransferase 10. Its related pathways are Other types of O-glycan biosynthesis and Biological oxidations. | 8.9             | 4.28E-05 | 0.019890845 |
| PRPSAP1 | Phosphoribosyl pyrophosphate synthetase-associated protein 1. This gene is related to magnesium ion binding and enzyme inhibitor activity. | 10              | 4.46E-05 | 0.019890845 |
| TLX3 | T-cell leukemia homeobox 3. Its related pathways are Transcriptional misregulation in cancer and SIDS Susceptibility Pathways. | 103.6           | 5.11E-05 | 0.021040208 |
| RP11-259G18.1 | Uncharacterized | 21.7            | 7.50E-05 | 0.024582733 |
| DAZ4 | Deleted in azoospermia 1. This gene is related to this gene include RNA binding and nucleotide binding. | 7               | 7.68E-05 | 0.024582733 |
| TMEM52 | Transmembrane protein 52 | 50.8            | 7.68E-05 | 0.024582733 |
| AC007879.2 | Uncharacterized | 13.3            | 7.93E-05 | 0.024582733 |
| PCMTD2 | Protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2. This gene is related to protein-L-isoaspartate (O-aspartate) O-methyltransferase activity. | 10.4            | 8.26E-05 | 0.024582733 |
| HNF1A-AS1 | HNF1A antisense RNA 1 affiliated with the antisense RNA class. | 13.6            | 8.86E-05 | 0.024984166 |
| SUV420H2_1 | Uncharacterized | 21.7            | 9.76E-05 | 0.026140534 |
| ATP6V1G2 | ATPase, H+ transporting, lysosomal 13 kDa, V1 subunit G2. A multisubunit enzyme that mediates acidification of intracellular compartments of eukaryotic cells. | 27.3            | 1.06E-04 | 0.027114541 |
| TCP10L | T-complex 10-like. An important paralog of this gene is TCP10L2. | 13.9            | 1.23E-04 | 0.028632706 |
| MCU | Mitochondrial calcium uniporter. It is related to calcium channel activity and uniporter activity. | 7.8             | 1.23E-04 | 0.028632706 |
| ZFHX3 | Zinc finger homeobox 3. Its related pathways are Transcriptional Regulatory Network in Embryonic Stem Cell. | 8               | 1.59E-04 | 0.035570625 |
| RP11-508 M8.1 | Uncharacterized | 10.7            | 1.85E-04 | 0.039536736 |
| CCL3 | Chemokine (C-C motif) ligand 3. Its related pathways are Signaling by GPCR and TGF-Beta Pathway. | 31              | 2.13E-04 | 0.043901777 |
| LL22NCO3-63E9.3 | Uncharacterized LOC648691, affiliated with the ncRNA class. | –22.2          | 2.30E-04 | 0.045120747 |
| RP11-180P8.3 | Uncharacterized | 46.1            | 2.36E-04 | 0.045120747 |
| RP11-213H15.3 | Uncharacterized | 11.1            | 2.56E-04 | 0.046011494 |

*AD000091.2, C11orf95, LPAR1, LBR, GNAI1, HOXD13, EARS2, PRDM13, RAB11FIP5, CTC-504A5.1, CHST10, PRPSAP1, TLX3, RP11-259G18.1, DAZ4, TMEM52, AC007879.2, PCMTD2, HNF1A-AS1, SUV420H2_1, ATP6V1G2, TCP10L, MCU, ZFHX3, RP11-508 M8.1, CCL3, LL22NCO3-63E9.3, RP11-180P8.3, RP11-213H15.3
signaling, p53 signaling, PI3K/AKT signaling, and acute phase response signaling.

**Discussion**

Unraveling the mechanism of spontaneous HCV clearance after an acute phase of infection in HIV infected individuals provides valuable information that will be informative for the development of a vaccine against HCV. In a genome-wide association study of HCV infected individuals, SNPs in the region of the *IL28B* gene, encoding interferon-λ-3, were shown to be associated with the outcome of HCV infection; the CC genotype enhances spontaneous resolution of HCV infection, whereas those with the TT genotype showed a great propensity to develop chronic HCV infection [15, 16]. In the current study we could not detect any significant relationship between CC genotype and HCV clearance, because the CC genotype was found in four of the five clearance subjects and four of the eight chronically HCV infected subjects. The distribution of the genotypes in the infected subjects suggests that other factors beside the *IL28B* SNPs play a role in the spontaneous HCV clearance in the co-infected individuals [14, 18, 27]. However, the small sample size in our study also could explain our failure to detect an association between *IL28B* polymorphisms and HCV clearance.

Recent advances in RNA sequencing technology have provided opportunities to comprehensively and quantitatively sequence RNA and examine virus-host interactions at the transcriptional level. Two studies have reported transcriptomes analysis of liver tissues of HCV infected chimpanzees with spontaneous viral clearance or chronic infection outcomes. Nanda et al [18] reported that early induction of the genes associated with cell proliferation and immune activation, especially interleukin binding factor 3 (ILF3) and cytotoxic granule associated RNA binding protein (TIA1), was associated with subsequent HCV clearance in infected chimpanzees. Suet et al [28] reported that cellular genes induced by IFN-γ and those involved in antigen processing/presentation and the adaptive immune response were associated with HCV clearance. These reports suggest that host innate/adaptive immune responses play an important role in HCV clearance. However, studies on the transcriptome of liver tissues from HIV/HCV co-infected individuals.

### Table 2

| DEGs in plasma before HCV infection in clearance group compared to those in chronically infected group (Continued) |
|-------------------------------------------------|
| **Gene ID** | **Description** | **Fold Change** | **P-value** |
| IL17D | Interleukin 17D. Its related pathways are IL-17 Family Signaling Pathways and IL-17 Family Signaling Pathways. | 40 | 2.58E-04 | 0.04601494 |
| AC016831.7 | Uncharacterized | 5.8 | 2.71E-04 | 0.04688832 |
| NFKBIL1 | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1 | 8 | 2.98E-04 | 0.049193252 |
| SLC2A6 | Solute carrier family 2, member 6. Its related pathways are PAK Pathway and HIF1Alpha Pathway. | 7.9 | 3.03E-04 | 0.049193252 |

*DEG Differentially expressed gene*
| DEGs          | Function                                                                 | Fold difference | \( P \) value | FDR          |
|--------------|---------------------------------------------------------------------------|-----------------|---------------|--------------|
| MIXL1        | Mix paired-like homeobox. Among its related pathways are cardiac progenitor differentiation and adipogenesis. | 142.7           | 3.52E-07      | 0.00142576   |
| SLC30A3      | Solute carrier family 30 (zinc transporter), member 3. Its related pathways are transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds and transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds. | 23.8            | 5.32E-07      | 0.00142576   |
| SFRPS        | Secreted frizzled-related protein 5. Its related pathways are Wnt signaling pathway (KEGG) (Wnt proteins are secreted morphogens that are required for basic developmental processes) and Non-Canonical Wnt Pathway. | 53.4            | 2.05E-06      | 0.00365475   |
| AC007879.2   | Uncharacterized                                                           | 13.8            | 1.45E-05      | 0.00595947   |
| AD000091.2   | Uncharacterized                                                           | 283.2           | 1.05E-05      | 0.00595947   |
| C12orf44     | Autophagy related 101                                                    | 18.9            | 8.91E-06      | 0.00595947   |
| HNF1A-AS1    | HNF1A antisense RNA 1 affiliated with the antisense RNA class.            | 48.4            | 1.32E-05      | 0.00595947   |
| LBR          | Lamin B receptor. Its related pathways are Metabolism and Metabolism.     | 70.9            | 1.36E-05      | 0.00595947   |
| MTMR1        | Myotubulin related protein 1. Its related pathways are Metabolism and Metabolism. | 20.4            | 1.44E-05      | 0.00595947   |
| NLRP13       | NLR family, pyrin domain containing 13                                   | 10.9            | 5.86E-06      | 0.00595947   |
| PPP1R3G      | Protein phosphatase 1, regulatory subunit 3G. It is related to protein phosphatase binding and glycogen binding. | 82.1            | 1.25E-05      | 0.00595947   |
| PRPSAP1      | Phosphoribosyl pyrophosphate synthetase-associated protein 1. This gene is related to magnesium ion binding and enzyme inhibitor activity. | 56.8            | 1.29E-05      | 0.00595947   |
| RAB11FIP5    | RAB11 family interacting protein 5 (class I). Its related pathways are Endocytosis and Delta508-CFTR traffic / ER-to-Golgi in CF. | 53.4            | 1.01E-05      | 0.00595947   |
| RP11-436F21.1| Uncharacterized                                                           | 9.6             | 1.93E-05      | 0.00740049   |
| C11orf95     | Chromosome 11 open reading frame 95. Diseases associated with C11orf95 include ependymoma and chondroid lipoma. | 119.4           | 2.56E-05      | 0.00913053   |
| HOXD13       | Homeobox D13. This gene is related to sequence-specific DNA binding transcription factor activity and chromatin binding. | 96              | 3.07E-05      | 0.01028157   |
| RP11-508M8.1 | Uncharacterized                                                           | 14.6            | 3.46E-05      | 0.0109017    |
| CTC-504AS.1  | Affiliated with the ncRNA class                                          | 9.9             | 3.82E-05      | 0.01135634   |
| IRS4         | Insulin receptor substrate 4. Its related pathways are Signaling by GPCR and Insulin receptor signaling cascade. | 454.4           | 5.62E-05      | 0.01466991   |
| MANEAL       | Mannosidase, endo-alpha-like. It is related to hydrolase activity.        | 14.7            | 5.95E-05      | 0.01466991   |
| MCU          | Mitochondrial calcium uniporter. It is related to calcium channel activity and uniporter activity. | 11.9            | 6.03E-05      | 0.01466991   |
| S1V420H2.1   | Uncharacterized                                                           | 18.4            | 5.71E-05      | 0.01466991   |
| SLC25A18     | Solute carrier family 25 (glutamate carrier), member 18. It is related to this gene include symporter activity. | 78.6            | 6.39E-05      | 0.01489091   |
| GNA11        | Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1. Its related pathways are GPCR Pathway and GPCR Pathway. | 10.8            | 6.69E-05      | 0.01492573   |
| ADPRHL1      | ADP-ribosylhydrolase like 1. It is related to magnesium ion binding and ADP-ribosylarginine hydrolase activity. | 7.3             | 9.39E-05      | 0.01676156   |
| CMTM4        | CKLF-like MARVEL transmembrane domain containing 4. It is related to cytokine activity. | 16              | 9.17E-05      | 0.01676156   |
| F10          | Coagulation factor X. It is related to the pathways of Hemostasis and Formation of Fibrin Clot (Clotting Cascade). | 11.4            | 8.76E-05      | 0.01676156   |
| LPAR1        | Lysophosphatidic acid receptor 1. Its related pathways are PI3K-Akt signaling pathway and Ras signaling pathway. | 9.6             | 8.50E-05      | 0.01676156   |
Table 3 DEGs in plasma after HCV infection in clearance group compared to those in chronically infected group (Continued)

| Gene Symbol/Ref | Description                                                                 | Log2 Fold Change | p-value  | q-value |
|-----------------|------------------------------------------------------------------------------|------------------|----------|---------|
| LYSMD4          | LysM, putative peptidoglycan-binding, domain containing 4                    | 10.3             | 8.30E-05 | 0.01676156 |
| RP11-886H22.1   | Uncharacterized                                                              | 9.3              | 9.38E-05 | 0.01676156 |
| ATP12A          | ATPase, H+/K+ transporting, nongastric, alpha polypeptide. It is related to the pathways of ion channel transport and Transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds. | 10.2             | 1.22E-04 | 0.01943478 |
| POU4F1-AS1      | RNF219 antisense RNA 1                                                       | 8.1              | 1.23E-04 | 0.01943478 |
| PRDM13          | PR domain containing 13. This gene related to methyltransferase activity.    | 9.3              | 1.23E-04 | 0.01943478 |
| ZXDA            | Zinc finger, X-linked, duplicated A. It is related to sequence-specific DNA binding transcription factor activity and C2H2 zinc finger domain binding. | 45.3             | 1.17E-04 | 0.01943478 |
| AC009495.4      | Uncharacterized                                                              | 8.9              | 1.59E-04 | 0.02368413 |
| RP11-410N8.1    | Uncharacterized                                                              | 29.8             | 1.55E-04 | 0.02368413 |
| ANKR3D4B        | Ankyrin repeat domain 34B                                                    | 10.4             | 2.00E-04 | 0.02675171 |
| DPY19L3         | Dpy-19-like 3. It is related to transferase activity, transferring glycosyl groups. | 5.2              | 2.08E-04 | 0.02675171 |
| LINC00609       | long intergenic non-protein coding RNA 609                                  | 8.9              | 2.08E-04 | 0.02675171 |
| RP11-180P8.3    | Uncharacterized                                                              | 96.5             | 2.15E-04 | 0.02675171 |
| RP11-402E3.2    | Uncharacterized                                                              | -8.3             | 2.05E-04 | 0.02675171 |
| TLX3            | T-cell leukemia homeobox 3. Its related pathways are Transcriptional misregulation in cancer and SIDS Susceptibility Pathways. | 83.1             | 1.96E-04 | 0.02675171 |
| ZFHX3           | Zinc finger homeobox 3. Its related pathways are Transcriptional Regulatory Network in Embryonic Stem Cell. | 12.5             | 2.11E-04 | 0.02675171 |
| SOHLH1          | Spermatogenesis and oogenesis specific basic helix-loop-helix 1. It is related to sequence-specific DNA binding transcription factor activity and protein dimerization activity. | 15.2             | 2.54E-04 | 0.03086759 |
| SLC51A          | Solute carrier family 51, alpha subunit. It is related the Bile secretion and Drug Induction of Bile Acid Pathway. | 7.6              | 2.60E-04 | 0.03093234 |
| TCF15           | Transcription factor 15 (basic helix-loop-helix). It is related to ERK Signaling and ERK Signaling pathways. | 8.8              | 2.74E-04 | 0.03191743 |
| RP11-118B18.1   | Uncharacterized                                                              | 6.5              | 3.08E-04 | 0.03504717 |
| ATP6V1G2        | ATPase, H+ transporting, lysosomal 13 kDa, VI subunit G2. A multisubunit enzyme that mediates acidification of intracellular compartments of eukaryotic cells. | 23.3             | 3.68E-04 | 0.04059745 |
| RP11-435M3.2    | Uncharacterized                                                              | 15.9             | 3.71E-04 | 0.04059745 |
| C4orf52         | A Protein Coding gene                                                         | 4.4              | 4.10E-04 | 0.04218642 |
| ZC3H8           | Zinc finger CCCH-type containing 8. It is related to sequence-specific DNA binding transcription factor activity and sequence-specific DNA binding. | 7                | 3.94E-04 | 0.04218642 |
| ZXDB            | A protein coding gene. It is related to nucleic acid binding.                 | 32.1             | 4.03E-04 | 0.04218642 |
| CHST10          | Carbohydrate sulfotransferase 10. Its related pathways are Other types of O-glycan biosynthesis and Biological oxidations. | 6.6              | 4.38E-04 | 0.04421968 |
| RP11-7F17.5     | Uncharacterized                                                              | -5.1             | 4.50E-04 | 0.04426996 |
| TMEM52          | Transmembrane protein 52                                                     | 108.9            | 4.55E-04 | 0.04426996 |
| NEURL           | A Protein Coding gene. It is related the pathways of Signaling by GPCR and Disease. | 6                | 4.68E-04 | 0.0447815 |
| AP3B1           | A protein coding gene. It is related to the pathway of Lysosome and Clathrin derived vesicle budding. | 7.8              | 5.28E-04 | 0.04960697 |

*DEG differentially expressed gene*
humans with different outcomes are very limited. Due to lack of available liver tissues, we focused on determining transcriptomes in plasma from HIV/HCV co-infected subjects with and without HCV clearance, since the quantity and characteristics of RNAs in plasma may reflect concurrent pathogenic changes in host liver tissues.

The availability of sequential plasma samples with defined onset and disease outcomes of HCV infection in highly characterized HIV-infected subjects from the MACS [25] provided us with a unique opportunity to identify, for the first time, the cellular transcripts that may be related to HCV clearance. Application of RNA sequencing technology in plasma has allowed us to determine such differentially expressed transcripts between subjects who cleared HCV and those who remained chronically infected after acute infection. In addition, the analysis of samples collected before and after acquisition of HCV infection provided information on cellular transcripts that may be associated with HCV clearance. Plasma transcriptome analysis of mRNA in subjects who cleared HCV and those who remained chronically HCV infected before and after HCV infection identified 12 DEGs that are uniquely expressed before HCV infection and a number of these genes and their upstream regulators are associated with innate immune response in the clearance group. For instance,
IL17D, a member of IL17 family, plays a major regulatory role in host defense and inflammatory diseases [29]. The NFKBIL1 gene encodes a divergent member of the I-kappa-B family of proteins and is involved in the regulation of innate immune response by negatively regulating TLR and interferon regulatory factor (IRF) signaling pathways. In addition, the NFKBIL1 gene has a role in negative regulation of transcriptional activation of NF-kB genes in response to pro-inflammatory stimuli [30]. CCL3, a chemokine, has been shown to increase NK activity [31]. This goes along with observation that subjects who resolved HCV infection had a higher frequency of HCV-specific interferon-gamma-producing T-cells (P = 0.017) and cytotoxic NK-cells (P = 0.005), compared to patients who became chronically infected [27]. In addition, in HCV infected patients treated with interferon-α and ribavirin, increases in MIP-1α, MIP-1β and RANTES levels after 24 h of treatment were exclusively observed in the group that showed a sustained virological response, suggesting an antiviral role of CCL3 [32]. Furthermore, IPA analysis suggests that cytokine signaling (such as IL-1, IL-4, IL-6, IL-8, IL-9, IL-10, TNFR1, interferon), TLR signaling, MAPK signaling, NF-kB signaling, communication between innate and adaptive immune cells, and ELF3 could be involved in HCV clearance.

**Conclusions**

Plasma RNA sequencing has identified the transcripts that are significantly associated with HCV clearance and are found to be expressed prior to infection. Some of the transcripts are involved in innate immune function. These results suggest that subjects who spontaneously clear HCV may have the transcripts that modulate forms of immunity that confer resistance to chronic infection with HCV. Further studies with longitudinal samples from HCV infected patients who cleared HCV infection, and those who became chronically infected may provide more definitive information about the nature of these inherited traits.
### Abbreviations

cDNA: Complementary DNA; DEG-A: Differentially expressed genes after HCV infection; DEG-B: Differentially expressed genes before HCV infection; DEGs: Differentially expressed genes; FDR: False Discovery Rate; HCV: Hepatitis C virus; HIV: Human Immunodeficiency virus; IL28B: Polymorphisms in interleukin 28B; ILF3: Interleukin binding factor 3; MACS: The Multicenter AIDS Cohort Study; TLR: Toll like receptor; TR1A: HCV clearance group after HCV infection; TR1B: HCV clearance group before HCV infection; TR2A: Chronically HCV infected group after HCV infection; TR2B: Chronically HCV infected group before HCV infection

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### Availability of data and materials

Data are held by the Center for Analysis and Management of the Multicenter AIDS Cohort Study (CAMACS). For access to the MACS data, please contact CAMACS at https://statepi.jhsphs.edu/macs/macs.html.

### Authors’ contributions

YC and PG conceived of the study. CS and DG provided assistance with experimental design and sample section. CR, ES, JM, VS, and OM contributed to the original manuscript. YC, CS, AA, SK and MD designed and performed the experimental design and sample section. CR, ES, JM, VS, and OM contributed with centers (principal investigators) at The Johns Hopkins Bloomberg School of Public Health (Joseph B. Margolick, Lisa P. Jacobson), Howard Brown Health Center, Feinberg School of Medicine, Northwestern University, and Cook County Bureau of Health Services (John P. Phair, Steven M. Wolinsky), University of California, Los Angeles (Roger Detels), and University of Pittsburgh (Charles R. Rinaldo).

### Competing interests

The authors declare that they have no competing interests.

### Consent for publication

Not applicable.

### Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB)/ethical standards committee in University of Pittsburgh, University of California, Los Angeles, Northwestern University and John Hopkins University. Written informed consent was obtained from all subjects participating in the study.

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