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***IFNL3* genotype is associated with differential induction of *IFNL3* in primary human hepatocytes**

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Abstract

Background—Lambda interferons (IFNLs) have potent antiviral activity against HCV, and polymorphisms within the *IFNL* gene cluster near the *IFNL3* gene strongly predict spontaneous- and treatment-related HCV infection outcomes. The mechanism(s) linking *IFNL* polymorphisms and HCV control is currently elusive.

Methods—IFNL induction was studied in primary human hepatocytes (PHH) from 18 human donors, peripheral blood mononuclear cells (PBMCs) from 18 human donors, multiple cell lines and induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-hepatocytes) from 7 human donors. After stimulation with intracellular RNA and infectious HCV, quantitative PCR (qPCR) primers and probes were designed to distinguish and quantify closely related IFNL messenger (m)RNAs from *IFNL1*, *IFNL2* and *IFNL3*.

Results—PHH demonstrated the most potent induction of IFNLs, although had lower pre-stimulation levels compared to PBMCs, monocytes and cell lines. PHH stimulation with cytoplasmic poly I:C induced >1,000-fold expression of IFNL1, IFNL2 and IFNL3. PHH from donors who were homozygous for the favourable *IFNL3* allele (*IFNL3*-CC) had higher IFNL3 induction compared to PHH from *IFNL3*-TT donors ($P=0.03$). Baseline IFNL mRNA expression and induction was also tested in iPSC-hepatocytes: iPSC-hepatocytes had significantly higher baseline expression of IFNLs compared to PHH ($P<0.0001$), and IFNL3 induction was marginally different in iPSC-hepatocytes by *IFNL* genotype ($P=0.07$).

Conclusions—Hepatocytes express IFNLs when stimulated by a synthetic viral RNA that signals the cell through the cytoplasm. IFNL induction may be greater in persons with the

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Disclosure statement

The authors declare no competing interests.

favourable IFNL3 allele. These data provide insight into the strong linkage between IFNL3 genetics and control of HCV infection.

Introduction

HCV has infected >185 million worldwide and 4 million individuals in the United States, leading to an increased risk of cirrhosis, hepatocellular carcinoma and liver failure [1]. Of those infected, only 14–30% of individuals spontaneously control acute HCV infection, whereas the majority of infected persons develop chronic hepatitis [2].

Spontaneous HCV control during acute infection has been associated with a number of host, viral and environmental factors [3,4]. The strongest genetic marker associated with spontaneous HCV control is a cluster of polymorphisms in close proximity to the genes for interferon lambdas (IFNLs) [5–7]. Interestingly, the same polymorphisms also strongly predict the responses of persons with chronic HCV infection to interferon-based treatment [8]. Interferon lambdas are type III interferons and comprise four distinct genes: *IFNL1*, *IFNL2*, *IFNL3* and the newly described *IFNL4* [9,10]. IFNLs share features with the type I interferons IFN α and IFN β that have been more extensively studied. IFNLs are strongly induced by double stranded RNA or viral infection via pathogen recognition receptors, namely Toll-like receptors (TLR) and RIG-like receptors, and have been shown to have a range of antiviral potency against numerous viruses *in vitro* [11].

The association with resolution of HCV infection maps to an approximately 15,000 base locus near the genes for *IFNL3* and *IFNL4* located on the long arm of chromosome 19 [4]. A single nucleotide polymorphism (SNP) at position rs12979860 is sufficient to mark the effect of the haplotype and has consistently predicted the outcome of acute infection and interferon treatment [5–8,12–14]. However, a dinucleotide polymorphism within the *IFNL4* gene is in strong linkage in Caucasians and appears to be better correlated in persons of African descent [9]. Paradoxically, the favourable genotype at *IFNL4* corresponds with failing to transcribe the gene. The genetic associations suggest that the lambda interferons may play a pivotal role in pathogenesis of HCV. However, the mechanism(s) linking HCV control with *IFNL3* genotype is not well understood.

A major hurdle in studying the basis of HCV control and *IFNL3* SNPs has been distinguishing the closely related IFNL2 and IFNL3 proteins, whose amino acid sequence is 96% identical [15,16]. The high degree of homology between the *IFNL2* and *IFNL3* genes, and consequently their relative messenger (m)RNA sequences, complicates quantification of the gene transcripts as well. Previous studies attempting to link the *IFNL3* genotype with IFNL expression measured a composite sum of IFNL2 and IFNL3 in peripheral blood mononuclear cells (PBMCs) or in homogenized liver and reported inconsistent findings [14,17,18]. In addition, it has been very hard to detect IFNL4 in any tissue including liver [9,10].

The present study was undertaken to discriminate IFNL3 from other IFNLs and a newly identified and related pseudogene. Furthermore, we sought to identify in which cell types the *IFNL3* genotype led to functional differences in the expression of IFNL3. IFNL4 could not

be reliably detected. The results of these investigations improve the tool kit for studying IFNLs and HCV infection, and add another link in the understanding of how the host response may constrain HCV replication.

Methods

IFNL template cDNA

Complementary DNA (cDNA) for *IFNL1* (*IL29*), *IFNL2* (*IL28A*) and the *IFNL3* (*IL28B*) were separately cloned into the pCR4-TOPO vectors (Thermo Fisher Scientific Open Biosystems Products, Huntsville, AL, USA) and were confirmed by direct sequencing. RNA was transcribed *in vitro* from cDNA templates using the Promega T7 Ribo MAX Express Large Scale RNA production system (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. The RNA was quantitated using NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA).

qRT-PCR assay to measure interferon lambda transcripts Primer/probe sequences for specific detection of *IFNL1*, *IFNL2* and the *IFNL3* were designed manually based on NCBI human genome assembly (NCBI36/hg18). Nucleotide sequences of the three genes and a nearby closely related pseudogene LOC728942 (Gene ID: 728942, discontinued on 20 June 2009; chr19:44417888-44420444) were aligned and nucleotide differences between the sequences were examined for presence of known polymorphisms based on single nucleotide polymorphism database (dbSNP) build 135 annotation. Fragments corresponding to exonic regions homologous in all of the IFNLs was selected to design locked nucleic acid (LNA) probes [19]. Primers and probes were synthesized by Integrated DNA Technologies Co. (Coralville, IA, USA; Additional file 1). House-keeping genes (beta-actin and HPRT1), albumin and interferon gamma-induced protein-10 (IP-10) were quantified using predesigned IDT probe-assays (Integrated DNA Technologies). Optimization of primer-probe concentrations and cycling conditions was performed with LightCycler 480 Probes Master (Roche Applied Science GmbH, Penzberg, Germany) on the LightCycler 480 II. C_t was calculated based on the mean of two house-keeping genes. Gene induction was compared between test conditions using the 2^{-C_t} method [20].

Total RNA was extracted and purified from cell lysates with Qiagen RNeasy plus spin columns (Qiagen, Valencia, CA, USA). First-strand cDNA was generated from total RNA using random hexamers and SuperScript III reverse transcriptase (Invitrogen, Grand Island, NY, USA).

Cell culture—PBMCs were thawed and maintained overnight prior to stimulation at a density of 2×10^6 cells/ml in RPMI 1640 (Sigma-Aldrich, St Louis, MO, USA), with 20% human serum (Sigma-Aldrich) and 10 mM HEPES buffer (Sigma-Aldrich) with 2 mM glutamine and 50 U/ml penicillin-streptomycin.

Cryopreserved primary human hepatocytes (PHH) from multiple normal human donors were purchased from Invitrogen. PHH were plated according to the manufacturer's instructions; briefly, 4–8 million cells in 1 ml storage medium were added to 48 ml of warmed thawing medium (CHRM® Medium, Invitrogen) and centrifuged at $100 \times g$ for 10 min at room

temperature. The resulting pellet was re-suspended in 4 ml of plating medium (Williams E Medium, phenol red free, with maintenance supplement pack and 10% FBS, Gibco, Gaithersburg, MD, USA). Cells were stained with trypan blue (Sigma–Aldrich), counted with a haemocytometer and then seeded at 20×10^4 cells/well in a 48-well collagen coated plate (CellDirect, Invitrogen). The cells were incubated with the plating medium at 37°C with 5% CO₂ for 4 h to allow the cells to adhere. The plating medium was then replaced with incubation medium overnight, prior to stimulation.

Cell stimulations were performed using polyinosinic-polycytidylic acid (poly I:C; Invivogen, San Diego, CA, USA; Catalogue Code: tlrl-pic) with or without the transfection reagent LyoVec™ (PolyI:C/LyoVec, Invivogen, Catalogue Code: tlrl-piclv).

Cell culture and hepatic differentiation of human induced pluripotent stem cell-derived hepatocyte-like cells in a 96-well plate format—All human induced pluripotent stem cells (iPSCs) including patient iPSC lines were cultured on the matrigel (BD) using the mTeSR (Stem Cell Technologies, Vancouver, BC, Canada) as described previously [21–23]. This study was done in accordance with Johns Hopkins ISCRO regulations and following a protocol approved by the Johns Hopkins IRB.

***IFNL3* polymorphism typing**—Genotyping of *IFNL3* single-nucleotide polymorphism rs12979860 and the *IFNL4* G SNP, rs368234815, were conducted using custom TaqMan® SNP Genotyping Assays from Applied Biosystems (Life Technologies, Carlsbad, CA, USA) performed according to manufacturer's instructions. The Roche LightCycler 480 Real-Time System (Roche Applied Science, Indianapolis, IN, USA) was used to amplify the DNA and measure the fluorescence from each reaction to determine each genotype as calculated by Endpoint Genotyping Software (Roche, Nutley, NJ, USA).

Human IFNL protein levels have been challenging to measure and discriminate between the different subtypes: human IFNL1/IFNL3 levels were measured using a commercially available EIA kit (R&D Systems Inc., Minneapolis, MN, USA; Cat. number DY1598B).

Statistical analysis

Statistically analysed data were analysed using *t*-tests or one-way ANOVA, depending on the experimental conditions and where appropriate. All results are presented as means $\pm$ standard deviation.

Results

Discrimination of IFNL subtype transcripts

LNA-probes were used to enhance the sensitivity and specificity of quantitative PCR (qPCR) assays for each IFNL subtype. A pseudogene (*IFNL3P1*) with sequence homology to the *IFNL* gene family was discovered 6 kb upstream of the *IFNL3* gene (Additional file 2). Serial dilutions of *in vitro* transcribed mRNA for IFNL1, IFNL2, IFNL3 and IFNL3P1 were quantified and each assay was found to be linear and efficient (Additional file 3). *In vitro* transcripts of IFNL1, IFNL2 and IFNL3 were detectable in small quantities and were able to be discriminated from each other when cross-titrated in up to 5 log₁₀ copies per assay

of interfering template (Additional file 4). *In vitro* transcripts of IFNL3P1 were not falsely detected in any of the reactions.

De novo and stimulated IFNL expression

mRNA expression of IFNL subtypes was measured in primary human cells and multiple cell lines (Figure 1). After normalizing expression to a panel of housekeeping genes, PBMCs and monocytes from multiple donors appeared to have the highest baseline expression of IFNLs. Intermediate levels of IFNLs were found in Huh 7.5.1, a hepatoma cell line, and HeLa and Raji cell lines. PHH had the lowest expression ($P=0.001$).

Since hepatocytes are the predominant cell type that harbours HCV infection and could directly sense intracellular viral nucleic acids, inducible IFNL expression was tested in human hepatocytes. PHH were incubated with various stimuli designed to recapitulate a viral infection and test for IFNL induction (Figure 2). Double-stranded HCV RNA is formed intracellularly as a viral replication intermediate and can be recognized by RIG-I/MDA-5 or by TLR3 [24,25]. Since RIG-I/MDA-5 is found in the cytoplasm, poly I:C was incubated with PHH using a LyoVec™ (PolyI:C/LyoVec; Invivogen) a liposomal agent to transfect the synthetic RNA into the cytoplasm. In contrast, since TLR3 is an endosomal molecule, poly I:C was also incubated without the transfection agent to allow endocytosis of the synthetic RNA. PHH that were stimulated with cytoplasmic poly I:C showed 100-fold higher induction of IFNL1, IFNL2 and IFNL3 compared to endosomal poly I:C ($P<0.001$; Figure 2 and Additional file 5). Although pre-conditioning with interferon alpha, but not interferon lambda, increased the amount of IFNL induction upon stimulation with endosomal poly I:C ($P=0.01$), pre-conditioning with interferons did not induce IFNLs to the levels found after stimulation with cytoplasmic poly I:C (Figure 2). IP-10, a chemokine that is robustly induced in chronic HCV infection and by type 1 interferons, was also up-regulated after stimulating PHH with poly I:C.

HCV RNA was also used to stimulate PHH: supernatants from a stable *in vitro* culture of the JFH-1 strain of HCV were taken from Huh 7.5.1 cells and incubated with PHH. In addition, sera from HCV-infected persons with high HCV RNA levels were incubated with PHH (Additional file 5). Surprisingly, incubation of HCV RNA failed to induce IFNLs and IP-10 to the level found after cytoplasmic poly I:C stimulation (Figure 2). Induction of IFNLs in PHH were noted at 6 h after stimulation and reached their maximum levels at 24 h (Figure 3). Consequently, for subsequent studies, PHH were stimulated with cytoplasmic poly I:C and IFNL induction was assessed between 6 and 24 h to understand the relationship between *IFNL3* genotype and cellular function.

PHH from 18 donors were genotyped at rs12979860, a signature marker of the predictive *IFNL3* haplotype in spontaneous and treatment-induced HCV control. In addition, the same PHH were genotyped at the newly described *IFNL4* G SNP (rs368234815) as it has also been associated with HCV control in people: there was 100% concordance between the *IFNL3* and *IFNL4* G SNPs (Table 1).

Baseline expression of IFNLs was similar for PHH before stimulation irrespective of *IFNL3* genotype (Figure 4A). PHH from persons who were homozygous for the favourable *IFNL3*

C allele at position rs12979860 (*IFNL3*-CC) showed a mean of 15.9-fold (95% CI ± 0.4) higher expression of IFNL3 at 6 h after stimulation with cytoplasmic poly I:C, compared to PHH from persons who were homozygous for the unfavourable T allele (*IFNL3*-TT; $P < 0.05$), and the difference was sustained at 24 h (Figure 4B). By 24 h, IFNL2 expression was also increased by 10.6 ± 0.4 -fold in PHH from *IFNL3*-CC donors compared to *IFNL3*-TT donors ($P = 0.04$). While the induction of IFNL1 and IP10 were consistent with these findings, they did not reach statistical significance. To test if IFNL production by other cell types was also inducible, PBMCs were stimulated with cytoplasmic poly I:C and IFNL mRNA was quantified after 24 h: IFNLs were inducible in PBMCs, but to a lesser degree than in PHH. In addition, there was no association between IFNL mRNA expression and *IFNL3* genotype in PBMCs (Additional file 6).

PHH from *IFNL3*-CC donors appeared to produce marginally more interferon lambda protein than PHH from *IFNL3*-CT and TT donors, but this was not significant. ($P = 0.5$; Additional file 7). Since the major phenotype assigned to the different *IFNL3* genotypes in humans is in the context of HCV replication, PHH were cultured with HCV using several previously reported techniques; however, we were unable to confirm productive replication of HCV.

IFNL induction in iPSC-derived hepatocyte-like cells

Human iPSCs have recently been shown to be differentiable into hepatocytes [21–23], and these iPSC-derived hepatocyte-like cells (iPSC-hepatocytes) also appear to support HCV replication [21,26]. To confirm the link between *IFNL3* genotype in hepatocytes and IFNL3 expression, hepatocyte-like cells were derived from genotyped iPSC lines (Table 1). The hepatic phenotypes and functionality of the hepatocyte-like cells derived from these human iPSC lines have been previously documented [21,23]. mRNA for the IFNL subtypes was measured at baseline and after stimulation with cytoplasmic poly I:C. Interestingly, baseline IFNLs were more abundantly expressed in iPSC-hepatocytes than in any other cell type (Figure 1). To compare genotypic differences, iPSC-hepatocytes derived from *IFNL*-CT and *IFNL*-TT donors were grouped together for comparison with *IFNL*-CC. While there was less induction of IFNLs in iPSC-hepatocytes compared to PHH, differential induction appeared to be consistent between the *IFNL3* genotypes; iPSC-hepatocytes from *IFNL*-CC donors had marginally higher induction of IFNL2 ($P = 0.054$) and IFNL3 ($P = 0.07$) compared to iPSC-hepatocytes from non-*IFNL*-CC donors 6 h after stimulation with transfected poly I:C, although these differences were less evident at 24 h (Figure 5).

Discussion

IFNLs are potent innate immune cytokines that, along with type 1 interferons, are the backbone of the early antiviral response. The epidemiological link between the *IFNL* gene locus and HCV control is among the strongest described in infectious diseases, but there have been few clues pointing to a mechanism. In this report we demonstrate that low constitutive expression of IFNLs by human hepatocytes can be markedly induced by a viral RNA analogue, in contrast to other cell types that have high baseline expression but little IFNL induction. In addition, genotypic differences in the *IFNL3* locus appear to be

correlated with differences in IFNL induction, and this finding may contribute to the difference in HCV control.

Several of the initial studies linking HCV outcome with *IFNL3* genotype demonstrated increased circulating IFNL protein in chronically infected patients with the favourable genotype, although different genetic loci were used in the studies [13,14]. Moreover, *IFNL3* genotype has been closely associated with first- and second-phase viral kinetics in HCV-infected patients receiving interferon-based therapy [27–31]. First-phase viral kinetics have been explained on the basis of inhibited viral production from infected cells, while second-phase kinetics suggest cell-mediated immune clearance of infected cells [32]. It appears evident from the present report that hepatocytes are at least one important inducible source of IFNLs. Indeed, Thomas *et al.* [33] have reported that HCV infection of hepatocytes results in potent expression of IFNLs that may account for the marked up-regulation of interferon stimulated genes (ISGs) in patients with chronic HCV infection.

A continued perplexing finding is that persistently HCV-infected persons with the favourable *IFNL3* or *IFNL4* genotypes largely, though not exclusively, have decreased hepatic ISG expression pre-treatment, while those with the unfavourable genotype have elevated pre-treatment hepatic ISG expression [34]. Notably, interferon treatment led to marked induction of ISGs in the former group compared to the latter group, suggesting that the capacity for ISG induction was more important than baseline ISG expression [34]. Future work will need to focus on reconciling baseline IFNL expression and *IFNL3* genotype *in vivo*, and to understand why the *IFNL3* genotype plays a major role in HCV but a lesser role in other chronic viral infections [35].

Since the majority of the implicated polymorphisms in the *IFNL3* gene locus are found near the upstream regulatory region of the gene, the present work suggests that innate immune sensing of HCV antigens and/or nucleic acids in the infected cell may result in altered downstream signalling in persons with the favourable versus the unfavourable *IFNL3* genotype. Altered signalling may lead to differential release of IFNLs that in turn leads to differences in ISG production, as seen here. Given the important antiviral role of induced ISGs, it is likely that HCV control is directly linked to the capacity of hepatocytes to up-regulate ISGs. In contrast, the unfavourable *IFNL4* *G* allele appears to encode a functional protein, while the favourable *IFNL4* allele does not result in a translated protein [9]. It is intriguing that the induction of IFNL2 and IP-10 mRNA demonstrated a trend toward being differentially expressed by *IFNL3* genotype, although these findings will need to be confirmed in larger sample sizes. We also uncovered an unexpected finding in persons heterozygous for the *IFNL3*-T allele: whereas in clinical studies persons with the *IFNL*-CT genotype tended to have unfavourable clinical outcomes, similar to persons with the *IFNL*-TT genotype, upon stimulation we found the induction of *IFNL2* and *3* in PHH from heterozygotes to be more comparable with PHH from persons who were homozygous for the *IFNL*-CC genotype. This discrepancy with published studies in bulk tissues may be because we did not study other human cells or tissues where *IFNL3* may also play a role.

There are several limitations in this study. We mainly focused on hepatocytes. *In vivo* the prevailing models suggest that while infection chiefly occurs in hepatocytes, other cells such

as plasmacytoid dendritic cells and natural killer T-cells play important roles [36]. However, others have confirmed that hepatocytes robustly produce type 3 interferons upon stimulation [33]. Another limitation was that the overall sample size of the study was relatively small, compared to larger GWAS studies. The present study was limited by primary cell availability. In addition, the majority of the PHH and iPSC-hepatocytes were derived from Caucasians; hence generalizing these findings will require testing in a larger number of PHH. Similarly, we did not study HCV replication directly, so while we infer that these results may pertain to HCV control, further studies will need to be pursued to make this mechanistic link. In addition, future research will focus on understanding how the underlying *IFNL* genotype determines the expression of IFNL mRNA and protein.

In closing, we describe sensitive and specific tools and apply them to demonstrate that hepatocytes can be induced to produce IFNLs that are differentially expressed by *IFNL3* genotype. These findings will inform future studies of both spontaneous and treatment-induced HCV control, given the importance of innate immunity to HCV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Baseline expression of *IFNL* subtypes

Messenger (m)RNA from the *IFNL* subtypes (*IFNL1*, *IFNL2* and *IFNL3*) and a closely located pseudogene (*IFNL3P1*) were quantified in primary human cells and cell lines before stimulation using quantitative PCR (qPCR) and specific primers and probes. Primary human hepatocytes (PHH) derived from 18 donors and peripheral blood mononuclear cells (PBMCs) derived from 18 donors. Monocytes were enriched from PBMCs using CD14+ selection. Induced pluripotent stem cells (iPSC)-hepatocytes were derived from iPSC cell lines of seven human donors. Cell lines for hepatocyte-like cells (Huh7.5.1 cells), B-lymphocytes (Raji cells) and epithelial cells (HeLa cells) were also studied. mRNA was quantified in cells after 24-h incubation in media without stimulation, and normalized to a panel of housekeeping genes. The y-axis indicates the log₁₀-fold difference between PHH and each of the tested cell types. ^aSignificant differences for a particular transcript between the tested cell type and PHH (independent t-test $P < 0.05$). Standard deviation bars are shown for biological replicates.

Figure 2. Induction of IFNL subtypes in PHH

Primary human hepatocytes (PHH) were stimulated with (left to right): cytoplasmic poly I:C (1,000 ng/ml with transfection reagent), endosomal poly I:C (1,000 ng/ml without transfection agent), pre-incubation with interferon (IFN) α protein (30 ng/ml) followed by endosomal poly I:C (1,000 ng/ml), pre-incubation with IFNL3 protein (30 ng/ml) followed by endosomal poly I:C (1,000 ng/ml), transfection reagent alone, serum from an HCV-infected individual and HCV+ (JFH1cc) supernatants from *in vitro* infection of Huh7.5.1 cells. The y-axis indicates log₁₀-fold changes between treated and untreated cells incubated for the same period of time. Messenger RNA for interferon gamma-induced protein-10 (IP-10; CXCL10) was also quantified in the same samples as a positive control. Two-way ANOVA using the Bonferroni post-test method was used to determine significant difference between testing conditions within each IFNL subtype: ^a $P < 0.001$, ^b $P < 0.01$.

Figure 3. Kinetics of IFNL induction in PHHs

Primary human hepatocytes (PHHs) were incubated for 6, 24 and 36 h with cytoplasmic poly I:C. The y-axis indicates log_{10} -fold changes between the treated cells and the untreated cells. Standard deviations are shown for eight biological replicates. IP-10, interferon gamma-induced protein-10.

Figure 4. *IFNL3* genotype and differential IFNL induction in PHH

IFNL messenger RNA was quantified in primary human hepatocytes (PHH) from donors with *IFNL3*-CC, -CT or -TT genotypes (A) at baseline (indicated by the cycle threshold [Ct] between the target transcript and the geometric mean of a panel of housekeeping genes) and (B) 6 or 24 h after induction with cytoplasmic poly I:C (expressed as log₁₀-fold change) between the target transcript in the treated cells compared to the untreated cells. ^a*P*<0.05 between *IFNL3* genotypes. IP-10, interferon gamma-induced protein-10.

Figure 5. IFNL genotype and differential IFNL induction in iPSC-hepatocytes

Messenger RNA induction of *IFNL* subtypes in induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-hepatocytes), stratified by *IFNL* genotype. iPSC-hepatocytes were derived from donors with rs129679860 CC and non-CC (CT or TT) genotypes, and stimulated with cytoplasmic poly I:C for 6 and 24 h. The y-axis indicates log₁₀-fold change in treated cells compared to untreated cells. IP-10, interferon gamma-induced protein-10.

Table 1

Characteristics of donors of primary hepatocytes used in this study

ID	Race	Gender	Age, years	<i>IFNL3</i> genotype (rs12979860)	<i>IFNL4- G</i> genotype (rs368234815)
PHH-1	Caucasian	Female	12	C/C	TT/TT
PHH-2	Caucasian	Female	48	C/C	TT/TT
PHH-3	Caucasian	Female	57	C/C	TT/TT
PHH-4	Caucasian	Female	57	C/C	TT/TT
PHH-5	Caucasian	Female	59	C/C	TT/TT
PHH-6	Caucasian	Male	44	C/C	TT/TT
PHH-7	Caucasian	Male	46	C/C	TT/TT
PHH-8	Caucasian	Male	53	C/C	TT/TT
PHH-9	Caucasian	Male	58	C/C	TT/TT
PHH-10	Caucasian	Male	65	C/C	TT/TT
PHH-11	Caucasian	Male	73	C/C	TT/TT
PHH-12	na	na	na	C/C	TT/TT
PHH-13	Caucasian	Female	31	C/T	TT/ G
PHH-14	Caucasian	Female	52	C/T	TT/ G
PHH-15	Caucasian	Male	61	C/T	TT/ G
PHH-16	na	na	na	C/T	TT/ G
PHH-17	Caucasian	Female	69	T/T	G/ G
PHH-18	Caucasian	Female	na	T/T	G/ G
iPSC-iAF8	Caucasian	Female	na	C/C	TT/TT
iPSC-iK1	Caucasian	Female	na	C/C	TT/TT
iPSC-iYF	Asian	Male	na	C/C	TT/TT
iPSC-iAF2	Caucasian	Male	na	C/T	TT/ G
iPSC-iH71	Caucasian	Male	na	C/T	TT/ G
iPSC-iH41	Caucasian	na	na	T/T	G/ G
iPSC-iH9N153	Caucasian	Female	na	T/T	G/ G

iPSC, human induced pluripotent stem cell; na, information is not available; PHH, primary human hepatocyte.