ORIGINAL ARTICLE

Genetic basis for variation in plasma IL-18 levels in persons with chronic hepatitis C virus and human immunodeficiency virus-1 infections

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INTRODUCTION

Inflammasomes are multi-protein cytosolic complexes that integrate several pathogen-triggered signaling cascades, ultimately leading to caspase 1 activation and the generation of pro-inflammatory cytokines including interleukin (IL)-1β and IL-1β (IL-1β).1-2 Although there are inflammasome-dependent and -independent means to induce mature IL-1β production,3-5 to date, inflammasome activation has been the only mechanism described to convert pro-IL-1β to mature IL-18.6 Thus, higher levels of IL-18 are indicative of inflammasome activation. Both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) are known to activate a specific inflammasome, the NOD-like receptor (NLR) family, pyrin domain containing 3 (NLRP3) inflammasome, as measured by increases in IL-18. For example, plasma IL-18 levels increase sharply during the acute phase of HCV infection, remain elevated in those with persistent infection, and return to the pre-infection level with spontaneous resolution of HCV.7 Similarly, levels are elevated in patients with chronic hepatitis and cirrhosis, and reduced after the infection is resolved either spontaneously or after antiviral treatment.8 IL-18 levels are also elevated in HIV infection, an effect that is partially reversed by antiretroviral therapy.9,10

Inflammasomes are multi-protein complexes integrating pathogen-triggered signaling leading to the generation of pro-inflammatory cytokines including interleukin-1β (IL-1β). Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections are associated with elevated IL-18, suggesting inflammasome activation. However, there is marked person-to-person variation in the inflammasome response to HCV and HIV. We hypothesized that host genetics may explain this variation. To test this, we analyzed the associations of plasma IL-18 levels and polymorphisms in 10 genes in the inflammasome cascade. About 1538 participants with active HIV and/or HCV infection in three ancestry groups are included. Samples were genotyped using the Illumina Omni 1-quad and Omni 2.5 arrays. Linear regression analyses were performed to test the association of variants with log IL-18 including HCV and HIV infection status, and HIV RNA in each ancestry group and then meta-analyzed. Eleven highly correlated single-nucleotide polymorphisms (r² = 0.98–1) in the IL-18-BCO2 region were significantly associated with log IL-18; each T allele of rs80011693 confers a decrease of 0.06 log pg ml⁻¹ of IL-18 after adjusting for covariates (rs80011693, rs111311302 B = −0.06, P-value = 2.7 × 10⁻⁹). In conclusion, genetic variation in IL-18 is associated with IL-18 production in response to HIV and HCV infection, and may explain variability in the inflammatory outcomes of chronic viral infections.

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genetic differences may partially explain variation in IL-18 response to viral infection. To investigate this hypothesis, we measured IL-18 levels in individuals with well-characterized chronic and active HIV viremia and/or HCV viremia, and evaluated the association of SNPs in 10 key genes in the viral-NLPR3 inflammasome activation pathway.

RESULTS
Participants
Demographic characteristics of the analyzed individuals are presented in Table 1. HIV-infected individuals were not under antiretroviral therapy. All individuals had their ancestry defined by principal component analysis using genetic variants. There were 386 individuals of EA, 796 of African ancestry (AA) and 356 of mixed or other ancestry. Age (P-value = 0.09) and sex (P-value = 0.05) were not associated with log IL-18.

Log plasma IL-18 by infection status
Log IL-18 was significantly higher in 450 persons with both HIV and HCV infections (mean = 2.76 log pg ml$^{-1}$, s.d. = 0.27 log pg ml$^{-1}$) than in the 688 HIV-monoinfected (mean = 2.61 log pg ml$^{-1}$, s.d. = 0.30 log pg ml$^{-1}$) and the 420 HCV-monoinfected participants (mean = 2.50 log pg ml$^{-1}$, s.d. = 0.30 log pg ml$^{-1}$), P-value = 2.2 x 10$^{-16}$ for the three-group comparison, Figure 1. Similar results were observed for each ancestry group independently (Supplementary Figure 1). The multivariable analysis after adjusting for the principal components demonstrates that the genetic differences may partially explain variation in IL-18 levels after adjusting for the principal components and the HIV RNA level of HIV RNA in plasma is also determinant of IL-18 levels.

Genetic association analysis
From 10 genes spanning a total of ~519 kb of DNA (Supplementary Table 2), we evaluated 721 genetic variants for EA, 1307 for AA and 1170 for mixed or other ancestry. We found a significant association of SNPs in 10 key genes in the viral-NLPR3 inflammasome activation pathway. The SNPs significantly associated with IL-18 levels after adjusting for the principal components and the HIV-HCV categories are shown in Figure 2. Table 3 displays their allelic frequency, effect size estimates and the annotation of the function and expression quantitative trait loci information described in public databases (https://genome.ucsc.edu/index).

Table 1. Descriptive statistics of the analyzed individuals by genetically estimated ancestry group

| Characteristic | Genetically estimated ancestry groups |
|---------------|--------------------------------------|
|               | European (n = 386) | African (n = 796) | Mixed or other (n = 356) |
| Female (n, %) | 283 (73.3)          | 703 (88.3)        | 334 (93.8) |
| Age (years), mean (s.d.) | 41.37 (11.5) | 40.85 (8.5) | 37.51 (9.4) |
| IL-18 (pg ml$^{-1}$), mean (s.d.) | 584.5 (547.4) | 546.0 (441.3) | 560.3 (410.0) |
| HIV–HCV infection status and HIV RNA | | | |
| HIV monoinfected (n, %) | 191 (49.5) | 156 (19.6) | 73 (20.5) |
| HIV monoinfected, low HIV RNA$^a$ (n, %) | 28 (7.25) | 104 (13.1) | 58 (16.29) |
| HIV monoinfected, moderate HIV RNA$^a$ (n, %) | 35 (9.06) | 102 (12.8) | 62 (17.4) |
| HCV monoinfected, high HIV RNA$^a$ (n, %) | 56 (14.5) | 149 (18.7) | 74 (20.78) |
| HIV–HCV co-infected, low HIV RNA (n, %) | 19 (4.92) | 75 (9.42) | 35 (9.83) |
| HIV–HCV co-infected, moderate HIV RNA (n, %) | 21 (5.44) | 76 (9.54) | 20 (5.61) |
| HIV–HCV co-infected, high HIV RNA (n, %) | 36 (9.32) | 134 (16.8) | 34 (9.55) |

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-18, interleukin-18; β, beta regression coefficient; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-18, interleukin-18; PC, principal component. *HIV low, medium and high RNA refers to 400–10 K, 10–50 K and > 50 K HIV copies per ml, respectively.

Table 2. Multivariate linear regression analysis of log IL-18 in all individuals

| Factor | β  | s.e. | P-value |
|--------|----|-----|---------|
| PC1    | 0.12 | 0.16 | 0.46    |
| PC2    | 0.24 | 0.16 | 0.13    |
| PC3    | -0.01 | 0.16 | 0.94    |
| PC4    | 0.19 | 0.16 | 0.25    |
| PC5    | -0.12 | 0.16 | 0.43    |
| HCV monoinfected, low HIV RNA$^a$ | -0.05 | 0.02 | 0.03    |
| HCV monoinfected, moderate HIV RNA$^a$ | 0.02 | 0.02 | 0.28    |
| HCV monoinfected, high HIV RNA$^a$ | 0.19 | 0.02 | 2 x 10$^{-16}$ |
| HCV–HCV co-infected, low HIV RNA | 0.15 | 0.02 | 7.0 x 10$^{-08}$ |
| HCV–HCV co-infected, moderate HIV RNA | 0.18 | 0.02 | 4.2 x 10$^{-10}$ |
| HCV–HCV co-infected, high HIV RNA | 0.27 | 0.02 | 2 x 10$^{-16}$ |

Abbreviations: β, beta regression coefficient; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-18, interleukin-18; PC, principal component. *HIV low, medium and high RNA refers to 400–10 K, 10–50 K and > 50 K HIV copies per ml, respectively.

Figure 1. Log plasma IL-18 by HCV and HIV infection status in all the individuals analyzed in the study. The thick horizontal line in each box plot represents the median for each category, the whiskers extend to 1.5 times the interquartile range from the box.
Figure 2. Association of variants in the IL-18-BCO2 region. The variants included 14 genotyped (squares) and 177 imputed SNPs (circles) within the region from 112 to 112.1 Mb on chromosome 11. The recombination in this region is plotted in the background in light blue. Pair-wise LDs between rs80011693 and other variants in IL-18-BCO2 were estimated using LD data in EUR population in the 1000 Genomes project (hg19/Nov 2014). The color from blue to red represents the $r^2$ values.

DISCUSSION

This investigation extends previous associations of genetic factors with IL-18 plasma levels by demonstrating that the variants in the IL-18-BCO2 region are important determinants of plasma IL-18 levels in the presence of a chronic viral infection. Prior studies demonstrated the importance of this region in persons without an ongoing infection.\textsuperscript{12–14} Compared to the SNPs, the greater magnitude of the effects ($\beta$ regression coefficient) associated with HIV monoinfection and HIV–HCV co-infection confirm the dominant role of viral infections explaining person-to-person differences in IL-18 levels.\textsuperscript{7,8} However, these dominant viral associations only accounted for a low percentage of the variance in IL-18 levels, suggesting that there are other unmeasured factors responsible for the person-to-person differences in IL-18 levels in each infection group. Our findings suggest that this genetic region has an important role in determining plasma IL-18 levels. It is also notable that in our multivariable model, no other non-genetic factor was as important, including ancestry.

The results of this and previous studies indicate a consistent locus effect in the IL-18 region represented by a SNP to SNP replication across studies. The association of genetic variants in IL-18 on plasma IL-18 levels was studied in older European adults included in the Cardiovascular Health Study (CHS)\textsuperscript{12} and European individuals in the In-CHIANTI study.\textsuperscript{13} They detected an association of IL-18 levels with rs1834481 and rs5744256, two intronic SNPs.
that were also significantly associated with IL-18 levels in our study. Likewise, rs1834481 was associated with IL-18 levels in a genome-wide association study of 1523 women of EA from the Nurses’ Health Study (NHS) and 435 from the Women’s Genome Health Study (WGHS). Moreover, rs5744256 and rs2043055 were previously associated with IL-18 levels in candidate gene studies in healthy individuals and in individuals with premature cardiovascular disease, respectively.

We looked at the publicly available RNA expression data to investigate the relationship of any of the associated SNPs or other SNPs with expression of IL-18 levels in diverse tissues. Of the 11 variants described in this study, 10 have been described as expression quantitative trait loci in tissues such as lung, pancreas and skin. Other SNPs not included in the present analysis, such as rs7020926, rs7030024, rs13283237, rs851038 and rs9443635, are described as related to IL-18 expression levels in circulating monocytes analyzed in 1490 unrelated European individuals, but no direct biological mechanism has yet been demonstrated.

**MATERIALS AND METHODS**

**Study participants**

A total of 1538 individuals were selected from five different study cohorts: the AIDS Link to the Intravenous Experience (ALIVE);23 Baltimore Before and After Acute Study of Hepatitis (BBAASH);24,25 Boston Area HCV Study Transmission, Immunity, Outcomes Network (BAHSTION);1,13 Correlates of Resolved Versus Low-Level Viremic Hepatitis C Infection in Blood Donors (REVELL Study);26 and Women’s Interagency HIV Study (WIHS),23 as previously described. The inclusion criteria were the availability of genome-wide genotyping data and evidence of active HCV and/or HIV infection as determined by presence of both antibodies and detectable RNA levels for more than 6 months prior to the date of IL-18 testing, as described previously.23,34 HCV- and/or HIV-infected individuals were not included in any anti-HCV or antiretroviral therapy at the time of sample withdraw. Each individual study obtained consent for genetic testing as approved by their governing Institutional Review Board and the Johns Hopkins School of Medicine Institutional Review Board.

IL-18 plasma levels

Mature IL-18 was measured in plasma from repositioned specimens using enzyme-linked immunosorbent assay (MBL International, Woburn, MA, USA) on a sample taken prior to antiretroviral therapy in the HIV-infected participants. The assay was performed per the manufacturer’s recommendations using 50 μl of plasma. Data were acquired using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA). The sensitivity of this assay is 12.5 pg ml⁻¹ and the average intra-assay coefficient of variation is 7.25%.

**Statistical analysis**

Plasma IL-18 levels, log-transformed for approximate normalization were the primary outcomes. HIV and HCV infection status, HIV RNA levels, genetic ancestry, gender and age were each included in the statistical model of linear regression to determine their effect on log IL-18. HIV and HCV infection status, and HIV RNA variables were modeled as a unique categorical variable that we refer to as ‘HIV–HCV infection, HIV RNA’. This variable groups the individuals into seven categories: HCV monoinfected, HIV monoinfected with low (400–10 K copies per ml), moderate (10–50 K copies per ml) and high HIV RNA levels (>50 K copies per ml), and co-infected with low, moderate and high HIV RNA levels. Analysis of variance was used to compare log IL-18 levels between HCV- and/or HIV-monoinfected, HIV mono- and HCV–HIV co-infected individuals with all individuals and by genetic ancestry.

**Genetic ancestry analysis**

Genetic ancestry was determined by principal components based on 10 340 unlinked SNPs across the genome using the smartpca program from the software package eigenstrat. Principal components analysis is used to summarize the background genetic variation of populations into a few variables that represent ancestral origin. Three distinct ancestry groups emerged including EA, AA and mixed or other ancestry, which likely includes Hispanic and Asian ancestry (Supplementary Figure 2).

**Genotyping and imputation**

The SNP genotyping data were obtained from a larger genome-wide association study4 using the Illumina Human Omni-Quad array for samples from the ALIVE, BBAASH, BAHSTION and REVELL study groups and using the Illumina Omni 2.5 array for the WIHS group. The selected SNPs are part of the NLRP3 inflammasome pathway and include the genes and 10 kilobases upstream and downstream of each gene. The pathway genes included were IL-18, IL-1B, activating signal cointegrator 1 complex
Genetic association analysis

Plink was used to analyze linear regression models adjusting for five principal components and HIV-HCV infection, HIV RNA. SNPs were modeled under an additive model in which genotypes are represented as 0, 1, 2 copies of the minor allele. Ancestry groups were tested separately and the results were meta-analyzed by combining ancestry-specific β (regression coefficient) estimates using a fixed effects inverse variance model implemented in META. LD (r²) was determined in each of the genetic regions by the ancestral group. This informed a modified Bonferroni correction using the effective number of independent SNPs (n = 103 SNPs for AA, n = 38 for EA and n = 57 for mixed or other ancestry, n = 197 total variants). Twenty-five of the 197 SNPs were common between at least two ancestry groups, and the remaining were unique. A modified Bonferroni corrected P-value < 2.9 × 10⁻⁴ was considered statistically significant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

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Supplementary Information accompanies this paper on Genes and Immunity website (http://www.nature.com/gene)