Genome-wide Association of NLRP3 Inflammasome Plasma Cytokines in Virally Suppressed People with HIV

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CROI Conference on Retroviruses and Opportunistic Infections

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BACKGROUND

- Even during suppressive antiretroviral therapy (ART), higher levels of immune activation and inflammation in people with HIV (PWH) may contribute to increased risk of morbidity and mortality compared to people without HIV (PWoH).[1-2]
- Signaling by the NOD-like receptor 3 (NLRP3) inflammasome (which induces IL-1β, IL-6, IL-18 production) has been associated with increased risk of vascular events[3-5] and mortality[6-7] in PWH on ART.
- There have been relatively few genome-wide association studies (GWAS) of HIV and most have focused on virologic outcomes.[8-9]
- Using whole genome sequencing (WGS), we performed a GWAS of plasma levels of NLRP3 inflammasome-induced cytokines (IL-1β, IL-6, IL-18) to identify potential novel mechanisms driving inflammation and immune activation in PWH on ART.

METHODS

- We performed WGS (~60X coverage) from 1002 virally suppressed (>1 year ART) PWH from the U.S. Military HIV Natural History study.[10]
- Circulating plasma concentrations of IL-1β, IL-6, and IL-18 were quantified via U-PLEX assay (Meso Scale).
- We performed quality control analyses to filter for heterozygosity, Hardy-Weinberg equilibrium, and chromosomal sex (PLINK[11]). Reads were aligned (joint variant calling using HaplotypeCaller[12]), mapped (using Picard[13] and GRCh38 assembly), and filtered by quality score (VQSR, GATK[12,14]).
- We performed single variant analyses using the GMMAT package,[15] and rare variant (gene-based) analyses using SMMAT.[16] We then estimated tissue-based gene expression levels for participants using the GTEx public database,[17] which allowed us to predict how tissue gene expression levels varied in relation to the NLRP3 inflammasome-induced cytokine levels using transcriptome-wide analyses (TWAS).
- All mixed-effect association models were adjusted for ancestry (first five ancestry principal components, as well as a genetic relatedness matrix), age, and sex.

- The NLRP3 inflammasome (which directly induces IL-1β, IL-6, IL-18) has been associated with increased risk of vascular events and mortality in PWH on ART.
- Whole genome sequencing of 1002 PWH on ART identified several DNA variants associated with host myeloid immune response, HIV replication, and cardiovascular risk to predict plasma NLRP3 inflammasome cytokine levels (IL-1β, IL-6, IL-18).
- Gene-based rare variant and transcriptome-wide association analyses supported the association with genes involved in host immunity and cardiovascular function and also predicted expression of these genes from key relevant tissues (e.g., coronary artery).

RESULTS

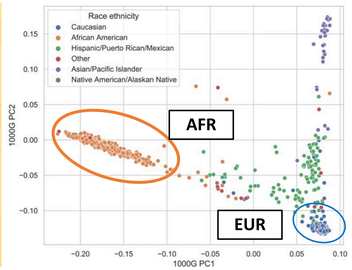
Figure 1: Characteristics of study population (N=1002 PWH on ART from the U.S. Military HIV Natural History cohort)

A.

Characteristic	Total
Age at HIV diagnosis (years)	31 (25 – 39)
Race/ethnicity (self-reported)	
Caucasian	429 (43.3%)
African American	410 (41.4%)
Other	161 (15.3%)
Sex at birth (% male)	918 (91.6%)
Cardiovascular risk factors	
Type II Diabetes	176 (17.6%)
Hyperlipidemia	670 (66.9%)
Hypertension	477 (47.6%)
Current Smoker	153 (15.5%)
Family history of CVD	45 (4.5%)
HIV clinical parameters	
Pre-ART Viral Load (log_{10} copies/mL)	4.55 (4.00 – 4.99)
Nadir CD4 count (cells/mm^3)	290 (193 – 388)

Medians with interquartile ranges or frequencies with percentages are shown. CVD = cardiovascular disease. ART = antiretroviral therapy.

B.



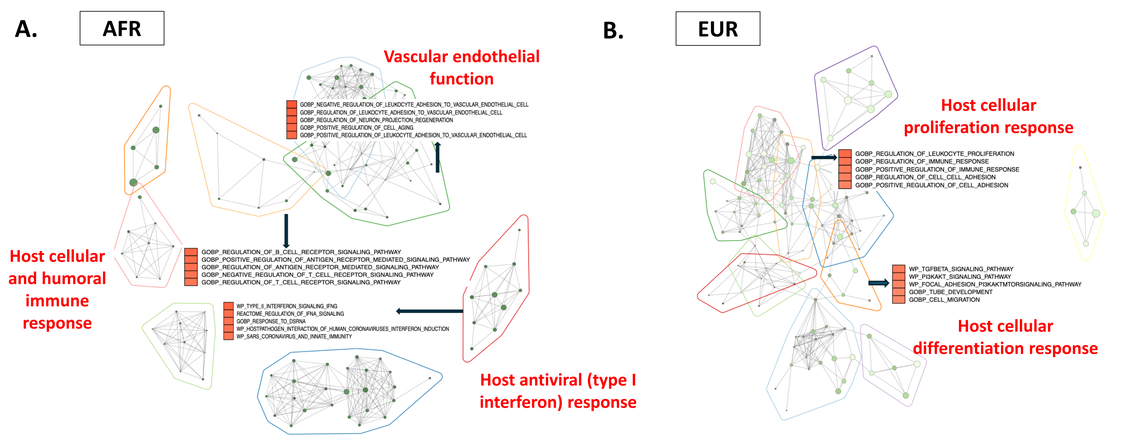
Population structure of N=1002 PWH on ART. Self-reported race/ ethnicity is shown by the colors in the legend. Principal component analysis using WGS data were used to define the two largest homogenous ancestry groups in our population (AFR, EUR) using the 1000 Genomes database.[18]

Figure 2: DNA variants in gene regions associated with atherogenesis, epithelial function, and host immune responses were associated with plasma IL-1β, IL-6, IL-18

Cytokine	Variant	Chr	Pos	Ref	Alt	Nearest Gene	Gene Name	MAF	P-value
AFR									
IL-1β	Novel	2	60161974	C	-	*BCL11A*	BCL11 Transcription Factor A	0.010	4.710e-9
IL-1β	rs2624337	2	90146357	C	T	*RPIA*	Ribose 5-Phosphate Isomerase A	0.015	4.567e-8
IL-1β	rs73680451	7	14580592	C	A	*DGKB*	Diacylglycerol Kinase Beta	0.010	7.071e-9
IL-1β	rs34129253	8	83757123	C	T	*RALYL*	RNA-Binding Raly-Like Protein	0.012	1.009e-8
IL-1β	rs10152564	15	94276135	T	C	*MCTP2*	Multiple C2 And Transmembrane Domain Containing 2	0.391	4.287e-8
IL-6	rs139154686	2	47317743	T	C	*CALM2, EPCAM*	Calmodulin 2, Epithelial Cell Adhesion Molecule	0.020	7.396e-9
IL-6	rs554755106	7	15426756	T	A	*AGMO*	Alkylglycerol Monooxygenase	0.018	4.026e-8
IL-6	rs57248810	12	92971792	T	C	*EEA1, NUDT4*	Early Endosome Antigen 1, Nudix Hydrolase 4	0.013	7.610e-9
IL-6	rs143821435	13	21903690	G	A	*FGF9*	Fibroblast Growth Factor 9	0.010	3.820e-9
IL-6	rs185742121	16	54162060	C	A	*FTO, IRX3*	Fat Mass And Obesity-Associated Protein, Iroquois Homeobox 3	0.017	1.186e-8
IL-6	rs142596565	20	7570072	T	C	*HAO1*	Hydroxyacid Oxidase 1	0.010	4.286e-9
EUR									
IL-6	rs78298311	2	23056093	T	C	*KLHL29*	Kelch Like Family Member 29	0.018	1.105e-8
IL-6	rs1374251239	15	23311393	C	T	*GOLGA6L1*	Golgin A6 Family Like 1	0.011	5.804e-10
IL-18	rs112466353	7	13991193	C	T	*ETV1*	ETS Variant 1	0.037	4.324e-8

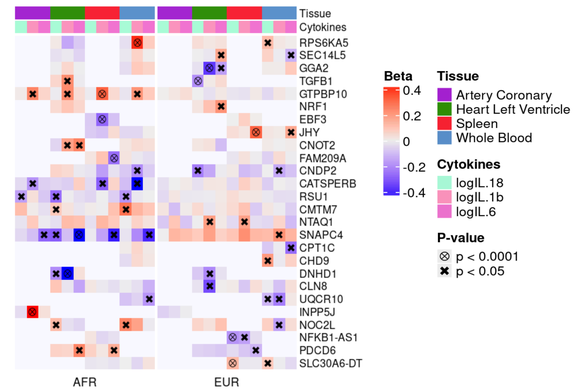
Top significant DNA variants associated with plasma cytokine levels, by ancestry population (AFR, EUR), at a genome-wide p-value of 5.0e-8. Variant = DNA variant rsID number. Chr = chromosome. Pos = chromosomal position. Ref = reference allele. Alt = alternate allele. MAF = minor allele frequency.

Figure 3: Rare variant gene-based analyses identified several immune pathways involved in vascular endothelial function, antiviral responses (e.g., type I interferon signaling), and host cellular and humoral responses to be associated with IL-1β



Gene set enrichment analyses for top significant genes associated with plasma IL-18 levels, by ancestry population (AFR, EUR). Similar results were observed for plasma IL-6 and IL-18 (data not shown). Only the gene sets enriched at p<0.05 are selected to compute an Overlap Network to identify the higher–order biological processes using visse.[19] MsigDB Hallmark, Gene Ontology, Curated gene set results were combined to identify gene pathways associated with plasma IL-18 levels. Size of nodes denotes the number of genes contributing to the gene set. Edges represent the overlaps based of the Jaccard index computed with a threshold of 0.2 . Heatmap shows top 5 enriched pathways in the cluster.

Figure 4: TWAS predicted increased expression of genes involved in lipid metabolism, T cell exhaustion, and NLRP3 inflammasome activation to be associated with plasma IL-1β, IL-6, and IL-18 levels from relevant tissues (e.g., coronary artery, spleen)



Heatmap of top genes expressed from tissues from the public GTEx database[17] that were associated with plasma NLRP3 inflammasome cytokine levels (p<0.05 labeled with an x; p<0.0001 labeled as an x with a circle). The strength of the associations are shown by the heatmap "Beta" by ancestry group (AFR and EUR on the x-axis), as well as by tissue type (top row of figure) and cytokine (second row of figure). Positive Beta values indicate an upregulation in gene expression.

CONCLUSIONS

- We identified several host genetic variants associated with NLRP3 inflammasome-induced cytokines in PWH on ART.
- Overall, these genetic variants fell into two categories, those involving the host immune response to HIV and pathways involved in cardiovascular disease pathogenesis.
- We identified 14 significant GWAS signals (p<5.0e-8) implicating host genes involved in myeloid immune responses (*ETV1*), HIV replication (*DDX17, DDX41, BCL11A*), HIV cell-cell transmission (*EEA1*), and cardiovascular disease risk (*KLHL29*), angiogenesis (*RNF213*), and cholesterol metabolism (e.g., *ABCA12, ABCC1, ABCA1, AGMO*).
- We further identified 3 expression quantitative trait loci (eQTLs) which were supported by published coronary artery tissue expression data (GTEx) using PrediXcan models.[17]
- We are currently performing Mendelian Randomization analyses to now link these findings to adjudicated incident vascular events from this cohort.

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NIH
- NIAID R01A133031
- NIAID Y1-AI-5072,
- U.S. DoD HU0001190002