Title

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Journal

AIDS, 30(11)

ISSN

0269-9370

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Publication Date

2016-07-17

DOI

10.1097/qad.0000000000001124

Peer reviewed

Host genetic predictors of the kynurenine pathway of tryptophan catabolism among treated HIV-infected Ugandans

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Objective: Plasma kynurenine/tryptophan ratio, a biomarker of indoleamine 2,3-dioxygenase-1 (IDO) activity, is a strong independent predictor of mortality in HIV-infected Ugandans initiating antiretroviral therapy (ART) and may play a key role in HIV pathogenesis. We performed a genome-wide study to identify potential host genetic determinants of kynurenine/tryptophan ratio in HIV-infected ART-suppressed Ugandans.

Design/methods: We performed genome-wide and exome array genotyping and measured plasma kynurenine/tryptophan ratio during the initial 6–12 months of suppressive ART in Ugandans. We evaluated more than 16 million single nucleotide polymorphisms in association with $\log_{10}$ kynurenine/tryptophan ratio using linear mixed models adjusted for cohort, sex, pregnancy, and ancestry.

Results: Among 597 Ugandans, 62% were woman, median age was 35, median baseline $CD4^+$ cell count was 135 cells/μl, and median baseline HIV-1 RNA was 5.1 $\log_{10}$ copies/ml. Several polymorphisms in candidate genes *TNF*, *IFNGR1*, and *TLR4* were associated with $\log_{10}$ kynurenine/tryptophan ratio ($P < 5.0 \times 10^{-5}$). An intergenic polymorphism between CSPG5 and ELP6 was genome-wide significant, whereas several others exhibited suggestive associations ($P < 5.0 \times 10^{-7}$), including genes encoding protein tyrosine phosphatases (PTPRM and PTPRN2) and the vitamin D metabolism gene, *CYP24A1*. Several of these single nucleotide polymorphisms were associated with markers of inflammation, coagulation, and monocyte activation, but did not replicate in a small US cohort ($N = 262$; 33% African-American).

Conclusion: Our findings highlight a potentially important role of IFN-γ, TNF-α, and Toll-like receptor signaling in determining IDO activity and subsequent mortality risk in HIV-infected ART-suppressed Ugandans. These results also identify potential novel pathways involved in IDO immunoregulation. Further studies are needed to confirm these findings in treated HIV-infected populations.

AIDS 2016, **30**:1807–1815

Keywords: antiretroviral therapy, genome-wide association study, HIV, kynurenine, tryptophan

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Received: 23 February 2016; revised: 10 April 2016; accepted: 11 April 2016.

DOI:10.1097/QAD.0000000000001124

Introduction

Despite effective antiretroviral therapy (ART), HIV-infected individuals experience higher rates of mortality and aging-associated diseases than HIV-uninfected controls [1,2]. Markers of immune activation predict clinical progression of disease, but even with ART suppression, immune activation levels fail to normalize [3,4]. To date, the specific biologic pathways driving persistent immune activation and its relationship to mortality remain unclear.

One proposed mechanism is the indoleamine 2,3-dioxygenase (IDO)-induced tryptophan catabolism pathway, which can be quantified as the ratio of kynurenine to tryptophan in plasma. IDO plays a critical role in autoimmune disorders [5], cancer [5], and fetal tolerance during pregnancy [5]. IDO is a key immunoregulatory enzyme that converts tryptophan to kynurenine [6]. Bacterial molecules such as lipopolysaccharide (LPS) and proinflammatory cytokines such as IFN-γ, TNF-α, and TGF-β promote increased IDO activity (Fig. 1) [7,8]. Several IDO-induced catabolites have been shown to decrease T-cell proliferation and function [9], and may compromise mucosal integrity, thereby increasing bacterial translocation and systemic immune activation [10]. These downstream effects may contribute to increased morbidity and mortality even during ART suppression [11].

Asymptomatic untreated HIV-infected participants have higher kynurenine/tryptophan ratios than HIV-uninfected controls, whereas HIV-infected participants with progression to AIDS have the highest kynurenine/tryptophan ratios [12]. Though IDO activity declines with ART, IDO has also been associated with a significant increase in mortality risk in Ugandans, both during untreated and treated HIV disease [11]. In a subsequent analysis of multiple immune markers in this same Ugandan cohort, kynurenine/tryptophan ratio was the strongest independent risk factor for mortality, even after adjusting for other markers of inflammation (IL-6), monocyte activation (sCD14), and $CD4^+$ or $CD8^+$ T-cell activation (%$CD38^+$HLA-DR^+) [13]. Kynurenine/tryptophan ratio has also been shown to predict mortality in ART-suppressed North Americans [3,4].

We performed a genome-wide association study (GWAS) of kynurenine/tryptophan ratio in HIV-infected Ugandans initiating ART. The goal of the study was to identify potential targets for intervention – perhaps in genes other than IDO itself – that may naturally (and presumably safely) influence IDO immunoregulation. We performed both a candidate gene (based on known factors that influence the IDO pathway) and genome-wide analysis. We found that single nucleotide polymorphisms (SNPs) in *TNF*, *IFNGR1*, and *TLR4* and SNPs in two additional pathways – one involving protein tyrosine phosphatase signaling and another related to vitamin D metabolism – are strongly associated with IDO activity. Several of the SNPs identified from these analyses were also associated with markers of monocyte activation (sCD14 and sCD163) and inflammation (IL-6 and D-dimer), additional biomarkers previously associated with increased risk of mortality during treated HIV disease. These findings provide further support that variation at these loci may play an important role in influencing the IDO pathway, as well as other interrelated immunologic pathways.

Materials and methods

Study participants

Participants were sampled from the Uganda AIDS rural treatment outcomes (UARTO) and anti-retrovirals for

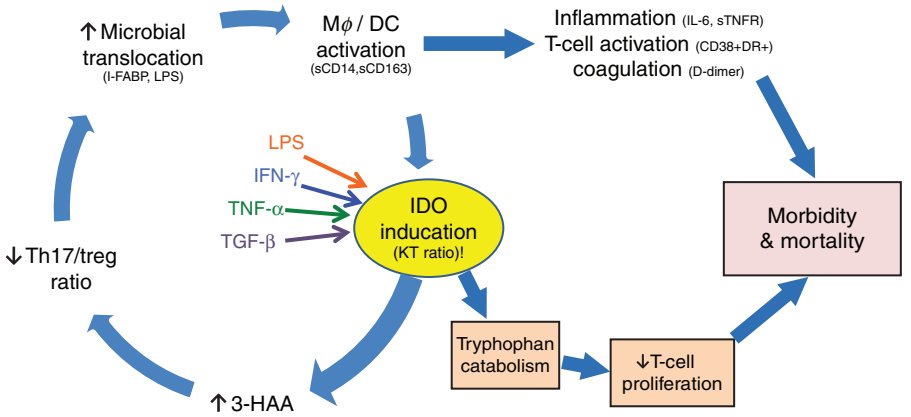

Fig. 1. Schematic illustrating the potential contribution of IDO to ongoing immune dysfunction and increased risk of morbidity and mortality during treated HIV infection. 3-HAA, 3-hydroxyanthranilic acid; DC, dendritic cell; IDO, indoleamine 2,3-dioxygenase; i-FABP, intestinal fatty acid binding protein; IFN-γ, interferon gamma; IL-6, interleukin-6; KT, kynurenine/tryptophan; LPS, lipopolysaccharide; MΦ, macrophage; sCD14, soluble CD14; sCD163, soluble CD163; sTNFR, soluble tumor necrosis factor receptor; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha.

Kaposi's sarcoma (ARKS) cohorts in Uganda. UARTO includes HIV-infected individuals initiating first ART at a rural clinic in Mbarara. ARKS includes HIV-infected participants with mild-to-moderate cutaneous Kaposi's sarcoma at a specialty clinic in Kampala. The primary ART regimen included two nucleoside reverse transcriptases (e.g. zidovudine/lamivudine, stavudine/lamivudine, or tenofovir/emtricitabine) and a nonnucleoside reverse transcriptase (e.g. nevirapine or efavirenz). Participants were included if they had sustained plasma HIV RNA less than 400 copies/ml [the lower limit of detection of an earlier Amplicor assay (Roche); the same cutoff was applied to a more recent Cobas Taqman assay (Roche) to maintain consistency], kynurenine/tryptophan ratio at month 6 and/or 12 of ART, and genotype data. ARKS participants were excluded for active untreated opportunistic infections or mucocutaneous Kaposi's sarcoma requiring chemotherapy. All participants provided written informed consent. This research was approved by the institutional review boards at each site and the University of California, San Francisco.

Phenotype and covariate data

Demographic and clinical data were collected at screening and follow-up visits approximately every 3 months. Data included self-reported pregnancy status and symptom reporting, as well as fasting morning laboratory measures (e.g., plasma HIV-1 RNA, $CD4^+$ T-cell counts, and archival of peripheral blood mononuclear cells), as described previously [11]. Plasma kynurenine/tryptophan ratio was measured in cryopreserved plasma using liquid chromatography–mass spectrometry/mass spectrometry [14] at pre-ART and approximately 6 and 12 months of ART. Mortality and follow-up were tracked via a limited vital status ascertainment program as described previously [15]. Cause of death was classified only in broad categories (e.g. illness, trauma, suicide, or related to childbirth). For this study, deaths due to trauma, injury, or suicide were excluded. Self-reported diagnoses of tuberculosis (TB) and/or TB medication history were adjudicated by reviewing clinical records and laboratory smear/culture results [16]. These data were only available for UARTO as TB diagnosis was an exclusion criterion for the ARKS study [16].

Genotyping and quality control

Genome-wide genotyping was performed at the RIKEN Center for Genomic Medicine using the Human Omni Express Bead Chip (Illumina, San Diego, California, USA), including over 700 000 SNPs. Additional whole exome genotyping of over 200 000 coding putative functional SNPs was performed at the University of California, San Francisco using the Axiom Whole Exome Array Chip (Affymetrix, Santa Clara, California, USA). A total of 928 participant samples were genotyped with the exome array and of these, 642 were also genotyped using a genome-wide array (Supplementary Fig. 1, http://links.lww.com/QAD/A918). After excluding samples due to ineligibility (lack of viral suppression), inadequate genotyping quality (<95% call rate), genetic relatedness (IBD > 0.125), and heterozygosity (±2 SD), 788 samples passed quality control for exome and/or genome-wide array data. Among these 788 samples with quality-controlled genetic data, 597 also had existing kynurenine/tryptophan ratio data (at month 6 and/or 12 of ART). We generated principal components using Eigensoft to include as covariates for ancestry in linear mixed effects models (http://genetics.med.harvard.edu/reich/Reich_Lab/Software.html) [17].

Genome-wide single nucleotide polymorphism imputation

Quality-controlled genotyping data were used to impute additional genotypes using IMPUTE2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html). We performed prephasing to estimate haplotypes prior to imputation, using SHAPEIT (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html). Only imputed SNPs with imputation quality score at least 0.80 were included in the final dataset.

Association analyses

We fit linear mixed effects regression models to test for associations between genotypes (coded as allele dosages) and $\log_{10}$ kynurenine/tryptophan ratios during month 6 and 12 of ART suppression. We restricted all analyses to ART-suppressed timepoints because SNPs associated with plasma kynurenine/tryptophan ratio prior to sustained ART suppression would mostly reflect genetic predictors of HIV viral load setpoint or $CD4^+$ T-cell count in these chronically HIV-infected participants initiating therapy. We also did not include HIV RNA levels or $CD4^+$ T-cell counts as covariates in the models as these factors could potentially be intermediates or colliders in a causal pathway between host genetics and plasma kynurenine/tryptophan ratio. Only SNPs with minor allele frequencies (MAF) more than 1% were included in the analyses. The final model included covariates for sex, pregnancy, and cohort (to account for Kaposi's sarcoma diagnosis [16] and other unmeasured cohort-specific differences), as well as the first 10 principal components to control for population stratification.

We performed candidate gene analyses on 17 307 SNPs in the following genes (including 100 kilobases flanking regions): *IDO1*, *IDO2*, *IFNG*, *IFNGR1*, *IFNGR2*, *TNF*, *TNFRSF1A*, *TGFB1*, *TGFBR1*, *TGFBR2*, *TGFBR3*, *IFNA1*, *IFNAR1*, *IFNB1*, *TLR4*, *TLR7*, *TLR8*, and *TLR9*. For the candidate gene analyses, we used a Bonferroni-adjusted significance threshold of P less than 5×10^{-5}, based on the number of SNPs tested. For the genome-wide analysis, we considered suggestive ($P < 5.0 \times 10^{-7}$) as well as genome-wide significant ($P < 5.0 \times 10^{-8}$) SNPs [18]. This is especially important in light of our limited sample size and allows for further investigation of potentially biologically relevant SNPs.

SNPs exhibiting suggestive or significant associations with kynurenine/tryptophan ratios were then tested for association with additional biomarkers (e.g., IL-6, D-dimer, and others) using linear mixed regression.

Replication analysis

We performed validation analyses among participants in the AIDS Clinical Trials Group (ACTG) studies NWCS 279 and NWCS 329 who had both GWAS [19] and kynurenine/tryptophan ratio data at 1 year of ART suppression [4]. Linear regressions were adjusted for ancestry, batch (for kynurenine/tryptophan ratio measurements), case-control status (occurrence of non-AIDS defining events) [4], and sex.

Results

Among 597 participants, 43% in ARKS and 70% in UARTO were woman, the median age was 35 years, median baseline $CD4^+$ T-cell count was 135 cells/μl, and median HIV RNA was 5.1 $\log_{10}$ copies/ml. At month 12 of ART, the median $CD4^+$ T-cell count was 266 cells/μl, and all participants had HIV RNA 2.6 $\log_{10}$ copies/ml or less (Table 1). Median kynurenine/tryptophan ratios in UARTO and ARKS were similar (63 versus 66 (nmol/l)/(μmol/l), $P = 0.93$). Variability (R^2) in kynurenine/tryptophan ratio at month 6 was 3.7% and at month 12 was 3.0%, and there was a statistically significant decline in plasma kynurenine/tryptophan ratio from month 6 to 12 of ART (69–60 (nmol/l)/(μmol/l), $P < 0.0001$) overall, as well as within individuals ($P < 0.0001$), consistent with previously published data [11].

By principal components analyses using 1000 Genomes reference data, our Ugandan population was most genetically related to Luhyans in Kenya (Supplementary Fig. 2, http://links.lww.com/QAD/A918). Q–Q plots of the discovery genome-wide association results gave an inflation factor of $\lambda = 1.04$, indicating that the results are not confounded by genetic ancestry (Supplementary Fig. 3, http://links.lww.com/QAD/A918).

Candidate gene single nucleotide polymorphisms and plasma kynurenine/tryptophan ratio

Several SNPs in the candidate genes *TNF*, *IFNGR1*, and *TLR4* were associated with kynurenine/tryptophan ratio at the Bonferroni-adjusted significance level of *P* less than 5.0×10^{-5}, conferring between a 1.11 and 1.71-fold increase in kynurenine/tryptophan ratio per increase in minor allele copy (Table 2). These included SNPs in the 5′ and 3′ regions of *TNF*, a SNP in *IFNGR1*, and a SNP in *TLR4*. SNPs strongly associated with kynurenine/tryptophan ratio included rs17200810 (3′ of *TNF*), which localizes to a region predicted to bind to several transcription factors, and rs276565 (5′ of *IFNGR1*) and rs270148 (intron 2 of *TLR4*), which are in active H3K27Ac histone mark regulatory regions (Supplementary Fig. 4, http://links.lww.com/QAD/A918). The SNP in the *IFNGR1* region also lies 100 kb upstream of *IL20RA* and 43 kb downstream of *IL22RA2*.

Genome-wide single nucleotide polymorphisms and plasma kynurenine/tryptophan ratio

Suggestive and significant SNPs from the genome-wide analysis were estimated to increase kynurenine/tryptophan ratio by 1.20–1.61-fold per increase in minor allele copy (Table 3). An intergenic SNP (rs56185965, 5′ of *CSPG5* and 3′ of *ELP6*) was significantly associated with kynurenine/tryptophan ratio at the genome-wide level ($P < 5.0 \times 10^{-8}$), whereas several SNPs exhibited suggestive associations (Fig. 2). Among the latter, two SNPs (rs6950107 and rs75257475) were in genes encoding two distinct protein tyrosine phosphatases; these SNPs localize to predicted transcription factor-binding sites for proteins involved in activator protein 1 signaling. A third SNP (rs13041834) was 67 kb upstream of *CYP24A1*, a gene encoding a key enzyme involved in vitamin D metabolism, and lies in an H3K27Ac histone mark-enriched region (Table 3, Supplementary Fig. 5, http://links.lww.com/QAD/A918). This SNP also lies 16 kb downstream of *BCAS1* (Breast Carcinoma Amplified Sequence 1), a gene that is overexpressed in breast cancer cell lines [20], and 18 kb downstream of *MIR4756*, a small noncoding RNA gene or microRNA, of unknown clinical significance [21].

Table 1. Characteristics of HIV-infected individuals initiating first antiretroviral therapy in the anti-retrovirals for Kaposi's sarcoma and Uganda AIDS rural treatment outcomes cohorts.

Characteristics	ARKS[a] ($N = 177$)	UARTO ($N = 420$)
Age	39 (29–40)	35 (29–40)
Female	76 (43%)	292 (70%)
Baseline $CD4^+$ cell count (cells/μl)	116 (25–250)	137 (80–206)
Proximal $CD4^+$ cell count (cells/μl)[b]	273 (171–465)	257 (175–257)
Baseline HIV RNA ($\log_{10}$ copies/ml)	5.2 (4.9–5.5)	5.1 (4.1–5.5)
Proximal HIV RNA ($\log_{10}$ copies/ml)	≤2.6	≤2.6
Plasma kynurenine/tryptophan ratio (nmol/l)/μmol/l)[c]	63 (50–93)	66 (50–86)

ARKS, anti-retrovirals for Kaposi's sarcoma; ART, antiretroviral therapy; UARTO, Uganda AIDS rural treatment outcomes cohort.
[a]Median and interquartile range shown except for sex (number and percentage).
[b]"Proximal" refers to the most recent $CD4^+$ T-cell count or HIV RNA relative to the most recent kynurenine/tryptophan ratio measurement.
[c]Plasma kynurenine/tryptophan ratio during months 6–12 of ART.

Table 2. Association of candidate gene polymorphisms with kynurenine/tryptophan ratio[a].

SNP[b]	Exp (beta)[c]	95% CI	*P*	Location	MAF	Gene
rs17200810[d]	1.65	(1.35, 2.02)	1.41×10^{-6}	3′	0.02	*TNF*
rs34451538	1.50	(1.26, 1.79)	3.90×10^{-6}	5′	0.03	*TNF*
rs276565[e]	1.11	(1.06, 1.16)	5.30×10^{-6}	5′	0.28	*IFNGR1*[f]
rs114064880	1.71	(1.36, 2.16)	5.33×10^{-6}	5′	0.01	*TNF*
rs11575838	1.68	(1.34, 2.11)	7.13×10^{-6}	3′	0.01	*TNF*
rs2770148[g]	1.15	(1.08, 1.23)	2.94×10^{-5}	Intron 2	0.12	*TLR4*

CI, confidence interval; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
[a]Linear mixed effects regression models of $\log_{10}$ plasma kynurenine/tryptophan ratio were adjusted for sex, pregnancy, cohort (ARKS versus UARTO), and the first ten principal components.
[b]Genotyped or imputed SNP.
[c]Fold change in kynurenine/tryptophan ratio (nmol/l)/(μmol/l) per copy of minor allele.
[d]Genotyped SNP, multiple transcription factors predicted to bind to this loci (e.g., IRF4, JUND; RUNX).
[e]Genotyped SNP, in active H3K27Ac mark regulatory region.
[f]This SNP also lies 100 kb upstream of IL20RA and 43 kb downstream of IL22RA2.
[g]In linkage disequilibrium with genotyped SNP, rs1413088.

Associations between selected single nucleotide polymorphisms and markers of inflammation and immune activation

To evaluate whether SNPs associated with kynurenine/tryptophan ratio might be related to additional immunologic pathways that have been associated with increased morbidity/mortality during treated HIV disease [4,11], we evaluated SNPs in relation to D-dimer, IL-6, sCD163, sCD14, and $CD4^+$ and $CD8^+$ T-cell activation levels during ART suppression (Supplementary Table 1, http://links.lww.com/QAD/A918). These biomarkers were only modestly correlated with each other ($R = 0.09$–0.45), with the exception of $CD4^+$ and $CD8^+$ T-cell activation ($R = 0.67$) (Supplementary Fig. 6, http://links.lww.com/QAD/A918). Several of the SNPs identified in the candidate gene analysis were associated with increased monocyte activation (sCD163 and sCD14) and increased IL-6 (Supplementary Table 1, http://links.lww.com/QAD/A918). SNPs identified in the genome-wide analysis were associated with sCD163, sCD14, D-dimer, and IL-6 (Supplementary Table 2, http://links.lww.com/QAD/A918).

Associations between selected single nucleotide polymorphisms and mortality

There were too few deaths in the cohort (35 deaths over a median of 7 years of follow-up) to directly perform a genome-wide analysis of predictors of mortality [11]. Nonetheless, we were able to assess whether SNPs associated with kynurenine/tryptophan ratio were also associated with mortality. We observed statistically significant associations with mortality and rs13041834 [adjusted hazard ratio = 1.82, 95% confidence interval (CI) = 1.06, 3.13] and rs75257475 (adjusted hazard ratio = 2.05, 95% CI = 1.12, 3.76) (Supplemental Table 3, http://links.lww.com/QAD/A918, Supplementary Fig. 7, http://links.lww.com/QAD/A918).

Sensitivity analyses to evaluate potential effect of nongenetic factors during early antiretroviral therapy

We had limited data on nongenetic factors, such as coinfections. However, we were able to perform posthoc analyses excluding 39 individuals with adjudicated TB diagnoses and found no significant change in the effect

Table 3. Association of polymorphisms with plasma kynurenine/tryptophan ratio from genome-wide analysis[a].

SNP[b]	Exp (beta)[c]	95% CI	*P*	Location	MAF	Nearest gene(s)
rs56185965	1.55	(1.33, 1.79)	8.73×10^{-9}	Intergenic	0.02	*CSPG5*; *ELP6*
rs6950107[d]	1.44	(1.26, 1.64)	7.14×10^{-8}	Intron 6	0.03	*PTPRN2*
rs17085469	1.20	(1.13, 1.29)	7.75×10^{-8}	5′	0.10	*RP11–736G13.1*
rs13041834[e]	1.17	(1.10, 1.25)	3.13×10^{-7}	Intergenic	0.12	*CYP24A1*; *BCAS1*; *MIR4756*
rs75257475[f]	1.23	(1.13, 1.32)	2.95×10^{-7}	Intron 1	0.07	*PTPRM*
rs200564710	1.30	(1.18, 1.44)	3.20×10^{-7}	Intergenic	0.05	*HOMER1*; *PAPD4*
rs114598920	1.37	(1.21, 1.54)	3.73×10^{-7}	Intron 1	0.03	*CLSTN2*
rs115718192	1.61	(1.34, 1.93)	4.13×10^{-7}	Intergenic	0.02	*TFRC*; *SDHAP1*
rs76101033	1.41	(1.23, 1.61)	4.29×10^{-7}	Intron 2	0.02	*RP11–6757F12.1*

CI, confidence interval; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
[a]Linear mixed effects regression models of $\log_{10}$ plasma kynurenine/tryptophan ratio were adjusted for sex, pregnancy, cohort (ARKS versus UARTO), and the first ten principal components.
[b]Genotyped or imputed SNP.
[c]Fold change in kynurenine/tryptophan ratio (nM/μM) per copy of minor allele.
[d]SNP predicted to decrease binding to SMAD3.
[e]SNP in active H3K27Ac mark region, in linkage disequilibrium with genotyped SNP, rs6064045.
[f]SNP predicted to alter binding to FOSL2, in linkage disequilibrium with 11 other SNPs with *P* less than 1×10^{-7}, including genotyped SNP rs1695225.

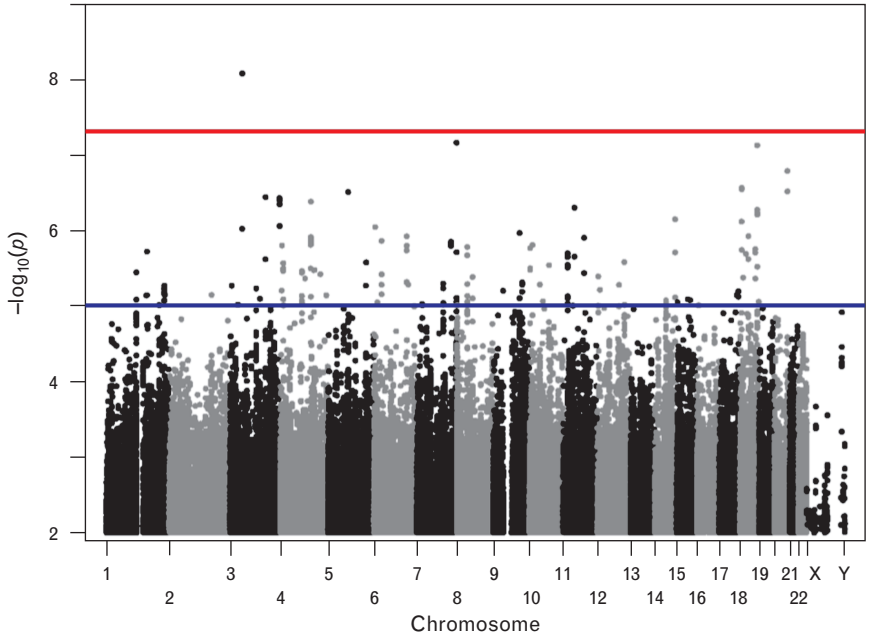

Fig. 2. Manhattan plot for association analyses. Genome-wide association test results are shown as-$\log_{10}$transformed *P* values for the linear mixed effects regression analyses of plasma $\log_{10}$ kynurenine/tryptophan ratio. Chromosomal location of single nucleotide polymorphisms is indicated on the *x*-axis. Single nucleotide polymorphisms significantly ($P < 5.0 \times 10^{-8}$) or suggestively ($P < 5.0 \times 10^{-5}$) associated with plasma $\log_{10}$ kynurenine/tryptophan ratio are noted above the red or blue line, respectively.

estimates – though exclusion of these participants reduced the sample size and significance of some associations (Supplementary Table 4, http://links.lww.com/QAD/A918). We also compared the associations between SNPs and kynurenine/tryptophan ratios at months 6 and 12 of ART separately to evaluate potential nongenetic influences (i.e. inflammatory conditions) that might increase kynurenine/tryptophan ratio during early, but not later, ART timepoints. Of the 597 participants, 586 had kynurenine/tryptophan ratio measurements at month 6 and 538 had kynurenine/tryptophan ratio measurements at month 12 of ART. The effect estimates were largely unchanged at month 12 compared with month 6 of ART (Supplementary Table 5, http://links.lww.com/QAD/A918).

Attempt to replicate findings in a non-African sample of convenience

We lacked resources to be able to measure kynurenine/tryptophan ratio in an additional African cohort. However, we had access to existing data from ACTG studies that included participants with genome-wide array [19] and kynurenine/tryptophan ratio data (at 1 year of ART suppression) [4] (Supplemental Table 6, http://links.lww.com/QAD/A918). Although this ACTG cohort was smaller than our Ugandan sample (262 versus 597 participants), only assessed kynurenine/tryptophan ratio at a single timepoint (as opposed to two timepoints in our Ugandan study), and had 33% participants with African ancestry (Supplementary Table 6, http://links.lww.com/QAD/A918), we evaluated whether the SNPs associated with kynurenine/tryptophan ratio in our Ugandan cohort might be replicated – but were unable to confirm these findings (Supplementary Tables 7 and 8, http://links.lww.com/QAD/A918).

Discussion

We performed a genome-wide study to identify potential host genetic determinants of kynurenine/tryptophan ratio in HIV-infected ART-suppressed Ugandans. A previous study in SIV-infected rhesus macaques of direct IDO and CTLA-4 inhibition led to severe autoimmune necrotizing pancreatitis in animals [22]. However, several phase I and II cancer treatment trials of direct IDO inhibition are currently underway [23]. The goal of the present study was to identify pathways that may modify IDO activity during treated HIV disease, potentially identifying targets for interventions other than IDO itself.

The specific candidate genes assessed in the study were selected by *a priori* knowledge of factors that modulate IDO in published in-vitro or animal model studies. IDO is upregulated in dendritic cells and phagocytes by proinflammatory cytokines including IFN-α, IFN-γ, TNF-α, and TGF-β [8,24]. Translocation of microbial products, including LPS, may stimulate Toll-like receptors, which then induce IDO activity [25]. The relative importance of these regulators *in vivo* during treated HIV infection, however, remains unclear.

We found that SNPs in *TNF*, *IFNGR1*, and *TLR4* were strongly associated with kynurenine/tryptophan ratio. Several of these SNPs localize to potential functionally relevant regions – for example, rs17200810 (*TNF*) lies in a region predicted to bind to several transcription factors involved in host immunoregulation, including the response to HIV (e.g. RELA [26], IRF4 [27], and JUND [28]). Both rs276565 (*IFNGR1*) and rs270148 (*TLR4*) lie in active H3K27Ac histone mark regulatory regions, loci that exhibit increased enhancer activity [29]. Interestingly, rs276565 (*IFNGR1*) also lies near *IL20RA*, which encodes for the IL-20 receptor (IL-20RA) and *IL22RA2*, which encodes for the soluble form of the IL-22 receptor (IL-22BP) (Supplementary Fig. 4B, http://links.lww.com/QAD/A918). Both IL-20 and IL-22 are members of the IL-20 subfamily of cytokines, which plays a critical role in enhancing the host innate immune response such as during viral infection or with intestinal epithelial homeostasis [30].

In the genome-wide analysis, we identified additional pathways that might plausibly be involved in IDO immunoregulation. PTPRN2 and PTPRM are both protein tyrosine phosphatases (PTPs), which, with protein tyrosine kinases, rapidly modulate signaling processes to maintain cell proliferation, differentiation, and gene transcription [31,32]. Based on ENCODE data, rs6950107 (*PTPRN2*) is predicted to decrease binding to SMAD3 [33], and rs75257475 (*PTPRM*) lies in a region that binds FOLS2 [34], a transcription factor that interacts with SMAD3 [35]. SMAD3 is a key intracellular effector of TGF-β signaling [36], and via formation of the activator protein 1 transcription factor complex [37] and binding to Foxp3 [38], plays a critical role in the development, function, and survival of regulatory T cells.

A second potential pathway identified from the genome-wide analysis is the vitamin D pathway. *CYP24A1* (rs13041834) encodes the key enzyme catalyzing the metabolism of active vitamin D. In mouse models, vitamin D inhibits T-cell proliferation [39], suppresses Th17 production [40], and induces regulatory T-cell expansion [41,42] – immunologic effects strikingly similar to those of IDO. Mice administered vitamin D also generates tolerogenic mature dendritic cells with enhanced *TGFB* and *IDO* mRNA expression, and increased IL-10 production with regulatory T-cell expansion [43]. In HIV-infected ART-suppressed patients, vitamin D supplementation is associated with decreased T-cell activation [44]. Of note, this SNP also lies downstream of *BCAS1* and *MIR4756*. However, the clinical significance of these genes and their association with IDO signaling are unknown.

We identified one genome-wide significant SNP, but to our knowledge, neither chondroitin sulfate proteoglycan a5 (*CSPG5*) nor elongator acetyltransferase complex subunit (*ELP6*) is biologically relevant molecules in IDO signaling. The SNP does not lie in a putative regulatory site, polymorphisms closer to these two genes were not associated with kynurenine/tryptophan ratio, and this SNP was not associated with other markers of immune dysfunction.

Our data are in contrast to a recent GWAS, which did not find any polymorphisms significantly associated with markers of immune activation and microbial translocation (sCD14 and i-FABP levels) [45]. However, only untreated HIV-infected individuals were included in the study, potentially making it more difficult to overcome additional variation due to the extent of disease progression and genetic and nongenetic contributors to viral load setpoint (factors that might confound the relationship between host genetics and sCD14). Participants were also restricted to those with higher $CD4^+$ T-cell counts, possibly excluding those with the most microbial translocation. Our study minimizes potential confounding by plasma HIV RNA levels by only evaluating ART-suppressed individuals. By sampling two different timepoints per participant, we also minimize within-subject variability and increase our power to detect an association with host genetic factors.

Our study has several limitations that deserve mention. First, we had limited data on nongenetic factors, such as coinfections. We were able to adjudicate potential TB diagnoses [16] and found little change in effect estimates after excluding these individuals. We also performed posthoc analyses of SNPs in relation to months 6 and 12 kynurenine/tryptophan ratios separately. These analyses did not demonstrate any bias toward a stronger relationship at month 6, which might suggest potential confounding by coinfection or other inflammatory conditions. Second, we had too few deaths in this cohort to directly perform a genome-wide analysis of mortality. Due to the lack of a dedicated mortality tracking system in Uganda, we also did not have information on the exact cause of death, for example, AIDS-related death. However, we did identify two SNPs that were significantly associated with mortality, suggesting that the genetic determinants of this pathway may be clinically meaningful. Third, though we included nearly 600 participants, based on a median of two observations per participant, we only had 80% power to detect SNPs that explain approximately 5% of the variance in plasma $\log_{10}$ kynurenine/tryptophan ratio (assuming a log-additive model, two-tailed Type I error rate of 5.0×10^{-8} and MAF $\geq 1\%$). This difference would be equivalent to a 2.0-fold difference in kynurenine/tryptophan ratio, corresponding to an estimated 3.7-fold increase in mortality risk [11]. Thus, it is likely that we lacked sufficient power, particularly for less common SNPs, to detect effect sizes that might be most clinically relevant. Finally, we did not have the resources to be able to measure kynurenine/tryptophan ratio in an African replication cohort. We instead analyzed existing ACTG

data but were unable to confirm the associations with kynurenine/tryptophan ratio observed in our Ugandan study. Based on the above estimates of power and the small number of participants with African ancestry in the replication cohort, however, we cannot definitively exclude the associations observed in our Ugandan study at this time.

In conclusion, we identified several polymorphisms associated with kynurenine/tryptophan ratio during treated HIV disease. Several of these SNPs lie in biologically plausible regions that could modulate the IDO pathway; these include polymorphisms in genes related to proinflammatory cytokines such as IFN-γ, TNF-α, and TGF-β, as well as genes involved in Toll-like receptor signaling and vitamin D metabolism. Although the SNP associations identified herein are plausible, in a small, primarily white cohort from a resource-rich region, we were unable to confirm these findings. The immunologic pathways predicting mortality among HIV-infected Ugandans may be different than those described in resource-rich settings; immune recovery (i.e. $CD4^+$ T-cell count) after ART initiation is significantly slower in resource-limited compared with resource-rich regions [15]. There may be nongenetic differences between regions that could plausibly affect kynurenine/tryptophan ratio such as viral clade (e.g. A and D in Uganda versus B in the United States) and prevalent coinfections. Further studies are needed to confirm the role of these SNPs in IDO-mediated gut epithelial immunity, especially among African populations.

Acknowledgements

The authors wish to acknowledge the participation of all the study participants who contributed to this work as well as the clinical research staff of the UARTO and ARKS cohorts who made this research possible. The authors would also like to acknowledge significant contributions by David R. Bangsberg, Yusuke Nakamura, Tricia H. Burdo, Kenneth C. Williams, Russell P. Tracy, Yap Boum, Miriam Laker-Oketta, Leslie W. Chinn, and Janine E. Micheli. Chin and Micheli coordinated DNA sample collection and extraction for genome-wide genotyping and assisted with initial genetic data collection. Bangsberg secured funding for and coordinated the design and operations of the UARTO cohort and Boum oversaw its operations locally. Laker-Oketta oversaw the local operations of the ARKS study. Nakamura coordinated the collection of the genotyping data at the RIKEN center. Burdo, Williams, and Tracy generated the additional biomarker data.

This work was supported in part by the National Institutes of Health (R56AI100765, R21AI078774, K24MH087227, T32AA007240, R01MH054907, UM1CA181255, P30AI27763, P01AI076174, and U01GM061390), the Doris Duke Charitable Foundation (P.W.H., Clinical Scientist Development Award 2008047), the Sullivan Family Foundation, HIV Translational Research Training Grant (T32AI060530) (S.A.L.), a Center for AIDS Research HIV Mentored Scientist Award (S.A.L.), Bristol-Myers Squibb Virology Fellowship Award (S.A.L.), and the NIH Pharmacogenomics Research Network-RIKEN Center for Genomic Medicine Global Alliance. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

S.A.L., P.W.H., and D.L.K. conceived and designed the study. J.N.M., P.W.H., and H.B. coordinated collection, management, and quality control processes for the Ugandan cohort clinical data and specimens. D.W.H., P.J.M., and P.W.H. coordinated collection of the ACTG replication cohort data. S.A.L., D.L.K., T.M., and M.K. coordinated generation of the genotyping data. Y.H. conducted the laboratory kynurenine/tryptophan ratio measurements. P.W.H. coordinated the acquisition of additional biomarker measurements. S.A.L. analyzed the data. J.A.M. and J.S.W. provided key data management and analysis support. S.A.L. wrote the report. All authors provided critical feedback in finalizing the report.

Conflicts of interest

There are no conflicts of interest.

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