2-1-2022

Age-associated gut dysbiosis, marked by loss of butyrogenic potential, correlates with altered plasma tryptophan metabolites in older people living with HIV.

Smita Ghare
University of Louisville

Richa Singhal
University of Louisville

Vaughn Bryant
University of Florida

Sabina Gautam
University of Louisville

Chanakya Charan Tirumala
University of Louisville

See next page for additional authors

Original Publication Information

Author:
Smita Ghare, Richa Singhal, Vaughn Bryant, et al

Publication:
JAIDS: Journal of Acquired Immune Deficiency Syndrome

Publisher:
Wolters Kluwer Health, Inc.

Date:
Feb 1, 2022

Age-Associated Gut Dysbiosis, Marked by Loss of Butyrogenic Potential, Correlates With Altered Plasma Tryptophan Metabolites in Older People Living With HIV

Smita Ghare, PhD, MSc,[a,b] Richa Singhal, PhD,[a,b] Vaughn Bryant, ScM,[c,d] Sabina Gautam, MBBS,[a,b] Chanakya Charan Tirumala, MD,[a,b] Praneet Kumar Srisailam, MD,[a,b] Andrea Reyes-Vega, MD,[a,b] Dushan Ghooray,[a,b] Craig J. McClain, MD,[a,b,e] Kristi Hoffman, PhD, MPH,[f,g] Joseph Petrosino, PhD,[f,g] Kendall Bryant, PhD,[h] Varan Govind, PhD,[i] Ronald Cohen, PhD,[c] Robert L. Cook, MD,[d] and Shirish Barve, PhD[a,b]

Background: Imbalance in tryptophan (TRP) metabolism and its neuroactive metabolites, serotonin and kynurenine (KYN), is a known pathogenic mechanism underlying neurocognitive impairment. Gut microbiota plays an important role in TRP metabolism, and the production of these neuroactive molecules affects neurocognitive function. Although both HIV infection and normal aging independently induce gut dysbiosis and influence TRP metabolism, their interactive effects on compositional/functional changes in gut microbiota and consequent alterations in TRP metabolites remain largely undetermined.

Methods: Older people living with HIV infection (PLWH, aged 50–70 years, n = 22) were enrolled in this cross-sectional pilot study. Metagenomic analysis of fecal microbiome using 16S Ribosomal ribonucleic acid gene sequencing and metabolomics analysis of plasma using mass spectrometry with a reverse-phase iquid chromatography tandem mass spectrometry were performed. Statistical analyses included the univariate linear regression and Spearman correlation analyses.

Results: Age-associated changes in plasma levels of key neuroactive TRP metabolites, serotonin and KYN, were seen in PLWH. Specifically, we observed age-dependent decreases in serotonin and increases in KYN and KYN-to-TRP ratio, indicative of dysfunctional TRP metabolism. Furthermore, the gut dysbiosis seen in older PLWH is characterized by a reduction of Firmicutes/Bacteroidetes ratio and butyrate-producing microbial families Lachnospiraceae and Lactobacillaceae. Of importance, correspondent with gut dysbiosis, increasing age was significantly associated with decreased plasma butyrate levels, which in turn correlated positively with serotonin and negatively with KYN/TRP ratio.

Conclusions: Age-dependent gut microbial dysbiosis distinguished by a decrease in butyrogenic potential is a key pathogenic feature associated with the shift in TRP metabolism from serotonin to KYN in older PLWH.

Key Words: aging, gut microbial dysbiosis, tryptophan metabolism, serotonin, kynurenine, butyrate, 16S rRNA sequencing, HIV, F/B ratio

(*J Acquir Immune Defic Syndr* 2022;89:S56–S64)

INTRODUCTION

In the era of Combination Antiretroviral Therapy, there has been a dramatic increase in the number of older people living with HIV (PLWH).[1–3] In the United States, more than 50% of the 1.3 million people with HIV are older than 50 years, and by the year 2030, it is estimated that this will increase to 70%. Although AIDS-defining illnesses have decreased, the prevalence of HIV-associated non-AIDS conditions remains high, particularly in aging individuals with long-standing HIV infection, indicating the onset of accelerated aging in PLWH.[4–6] Some of the significant comorbid conditions that develop during the normal course of the aging process, such as neurological, cardiovascular, hepatic, and physical frailty, are observed at a relatively earlier age in PLWH.[2,7,8] In particular, in relation to premature aging and neurological defects in PLWH, neurocognitive impairment has been reported to be present at higher rates in individuals older than 50 years.[9–12]

Several studies have documented the role of gut-derived metabolites in altering neurobiological functions. In this regard, metabolites derived from tryptophan (TRP) metabolism, ie, serotonin and kynurenine (KYN) function

Received for publication September 27, 2021; accepted October 1, 2021.

From the [a]Department of Medicine, University of Louisville, KY; [b] Alcohol Research Center, University of Louisville, KY; [c]Department of Epidemiology, Center for Cognitive Aging and Memory, Gainesville, University of Florida, FL; [d]Department of Clinical and Health Psychology, Center for Cognitive Aging and Memory, Gainesville, University of Florida, FL; [e]Robley Rex VAMC, Louisville, KY; [f]Department of Molecular Virology and Microbiology, Baylor College of Medicine; [g]Baylor College of Medicine Center for Metagenomics and Microbiome Research; [h]National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD; and [i]Department of Radiology, University of Miami, FL.

Supported by funding from R01AG061065, P20GM113226, and P50AA024337.

The authors have no conflicts of interest to disclose.

Correspondence to: Shirish Barve, PhD, Departments of Medicine and Pharmacology and Toxicology, University of Louisville Health Sciences Center, 505 S. Hancock St. CTR Building, Room 515, Louisville, KY 40202 (e-mail: shirish.barve@louisville.edu).

as neuroactive signaling molecules that affect neurocognitive function.[13–15] Indeed, dysregulated TRP metabolism is observed in various neurological disease conditions associated with aging and HIV-1 infection.[16–19] Serotonin [5-hydroxytryptamine (5-HT)], one of the well-studied neuroactive TRP metabolites, is primarily synthesized and stored in enterochromaffin cells (ECs), a specialized subtype of an intestinal epithelial cell.[14,20,21] The 5-HT biosynthesis occurs through the Trp hydroxylase 1 enzyme, which produces 5-hydroxytryptophan, which is further metabolized into 5-HT.[22] A decline in 5-HT levels has been observed during normal aging and in individuals with HIV-1 infection.[23,24] TRP metabolism also yields neuroactive metabolite KYN, which contributes negatively to neurocognitive outcomes.[17,25] The rate of TRP metabolism along the KYN pathway is dependent on the expression and activity of indoleamine 2,3-dioxygenase (IDO).[26] Apart from exerting negative effects on neurobiological function, KYN and its derivatives also contribute to peripheral inflammation and oxidative stress.[27] Systemic TRP and KYN levels change along with an increase in KYN-to-TRP ratio on aging and in age-related diseases and in HIV-infected individuals.[28–30]

There is gathering evidence that the gut microbiome participates in the regulation of not only the gastrointestinal and peripheral physiology but also the central nervous system function by modulating signaling pathways along the microbiota–gut–brain axis.[31,32] Accordingly, there is increasing emphasis on understanding the microbial management of TRP metabolism and production of neuroactive metabolites, ie, 5-HT and KYN that can affect neurological functions. Of importance, gut microbial dysbiosis and dysfunctional TRP metabolism both occur during the course of aging and HIV-1 infection.[33–35] Hence, understanding the interactive effects between aging and HIV-1 infection on the compositional and functional features of gut microbial dysbiosis that correlate with alterations in the levels of neuroactive TRP metabolites could provide relevant clinical insights into adverse neurocognitive processes that develop in older PLWH. Hence, this cross-sectional pilot study examined the status of neuroactive TRP metabolites, particularly serotonin and KYN, in association with gut microbial compositional and functional changes in older (aged 50–70 years) PLWH.

METHODS

Study Population

This was a cross-sectional pilot study of PLWH managed at the HIV Care Clinic at the University of Louisville. All procedures were in accordance with the ethical standards of the Helsinki Declaration (1964, 2008 amendment) of the World Medical Association and were approved by the University of Louisville Institutional Review Board (IRB# 08.0188). The HIV group included patients (n = 22) with an established diagnosis of HIV. All HIV-positive patients were on antiretroviral therapy (ART) and had controlled viral load (HIV RNA $<$ 400 copies/mL). Trained personnel collected clinical data from patient medical records and entered these data into a secure, web-based data management system hosted by the University of Louisville.

Sample Collection

Fecal and blood samples were collected under University of Louisville IRB-approved protocol (IRB # 08.0188) for metagenomic and metabolomic studies, respectively. Participants were informed about the fecal sample collection before their visit and thus were able to provide both fecal material and blood sample at the same time during the visit. Both fecal and blood/plasma samples were collected concurrently in the morning (between 9 and 11 AM) and subsequently stored in aliquots at −80°C. Fecal samples were collected using stool nucleic acid collection and preservation system kit as per manufacturer's instructions (Norgen Biotek Corp., Thorold, Canada) and later processed for metagenomic analyses as described further. The plasma samples were used to perform analysis of (1) TRP metabolites, including TRP, serotonin, and KYN, and (2) short chain fatty acid—butyrate, as detailed further. All participants enrolled in the study provided self-reported diet history and completed the "Food Frequency Questionnaire" at the time of enrollment. The study subjects with current antibiotic and/or probiotic use at the time of enrollment were excluded from the study.

Quantitative Analysis of TRP Metabolites

The quantitative metabolomics profiling on plasma samples was performed. In brief, butyrate levels were detected using a reverse-phase liquid chromatography (LC) tandem mass spectrometry (MS) custom assay as described earlier.[36] Ice-cold methanol–precipitated plasma samples were centrifuged at 13000*g* for 20 minutes. The 3-nitrophenylhydrazine reagent was added to 50 μL of supernatant and incubated for 2 hours. BHT stabilizer and water were mixed with samples before LC-MS injection. TRP metabolites were detected by combining the direct injection mass spectrometry (MxP500 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) with a reverse-phase LC-MS/MS Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria).[37] Plasma samples were dried with nitrogen, reprivatized with 5% solution of phenyl isothiocyanate, and metabolites were extracted with the addition of 300 μL methanol containing 5 mM ammonium acetate. Mass spectrometric analysis for butyrate was performed on an ABSciex 4000 (Arc Scientific, Boston, MA) and for TRP metabolites on API5500 Qtrap tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA). A targeted profiling scheme was used to quantitatively screen for metabolites using multiple reaction monitoring, neutral loss, and precursor ion scan.

The 16S Ribosomal ribonucleic acid (rRNA) Gene Sequencing

The 16S rRNA gene sequencing methods were adapted from those developed for the Human and Earth Microbiome Projects.[38–40] In brief, the fecal samples obtained from older PLWH study participants were used to extract total bacterial genomic DNA using the MagAttract PowerSoil Kit (Qiagen, Redwood City, CA). The 16Sv4 region was amplified by polymerase chain reaction and sequenced on the MiSeq platform (Illumina, San Diego, CA) using a 2 × 250-bp paired-end protocol, yielding paired-end reads that overlap almost completely.[41] In addition, the sequence reads were demultiplexed, denoised using the Deblur algorithm,[42] and assigned into operational taxonomic units at a similarity of 97% using the latest current SILVA database[43] containing only sequences from the v4 region of the 16S rRNA gene to determine taxonomies using usearch70 "usearch_global" function.[44] Biome file was generated for phylogeny information by aligning the centroid sequences with Multiple Alignment using Fast Fourier Transform[45] and creating a tree through FastTree.[46] The biome file was summarized, recording the number of reads per sample, and merged with a file that was generated for the overall read statistics, to produce a final summary file with readings, statistics, and taxonomy information.

Statistical Analyses

Descriptive statistics were calculated to describe the study samples with means and standard deviations (mean ± SD) for continuous variables and frequencies and percentages for all categorical variables. Correlations between parameters measured were calculated using age as a continuous variable in the univariate linear regression analysis.

Correlations between metabolites were calculated by Spearman correlation analysis. The significance level α was set at 0.05. Graphpad Prism software, version 8.03, was used to analyze all data sets.

RESULTS

Clinical Characteristics and Demographics of Older Adults Living With HIV

A total of 22 older PLWH with a median age of 56 years (mean ± SD 57.50 ± 4.02, range 52–69) were enrolled in a cross-sectional pilot study. Table 1 outlines the main characteristics of the study population. There were 20 men (90.91%) and 2 women (9.09%) with no prominent history of antibiotic and probiotic use. The evaluation of alcohol drinking was performed using Alcohol Use Disorders Identification Test criteria, and 17 participants (77%) showed minimal to no social drinking habits for alcohol, whereas 5 participants (23%) reported heavy alcohol drinking. All the participants were on ART and virally suppressed with a viral load less than 400 copies/mL (mean ± SD 150.00 ± 215.75). The $CD4^+$ T-cell count was 602.82 ± 437.68 cells/μL, and the $CD8^+$ T-cell count was 913.49 ± 416.90 cells/μL (Table 1).

TABLE 1. Demographic and Clinical Characteristics of PLWH

Samples	n = 22
Demographics	
Age, yrs	57.50 ± 4.02
Ethnicity/racial distribution, n (%)	
Non-Hispanic White	10 (45)
Non-Hispanic African American	10 (45)
Hispanic White	2 (9)
Hispanic African American	0 (0)
Sex	
Male	20
Female	2
AUDIT-C (alcohol measure)	
AUDIT-C score (0–4)	17 (77%)
AUDIT-C score (5–7)	5 (23%)
HIV infection history	
CD4+ T-cell counts (cells/μL)	602.82 ± 437.68
$CD8^+$ T-cell counts (cells/μL)	913.49 ± 416.90
CD4+/CD8+ T-cell ratio	1.34 ± 3.10
No. of participants with HIV viral load (>20 copies/mL) VL: 150.00 ± 215.75	5
No. of participants with HIV viral load (<20 copies/mL)	17
Anti-Retroviral treatment (n)	22
Neuromodulators (μM)	
Butyrate	0.840 ± 0.219
Serotonin	0.153 ± 0.199
Kynurenine	1.623 ± 0.639
Tryptophan	71.45 ± 18.22
Kynurenine/tryptophan ratio	0.0241 ± 0.011
Serotonin/tryptophan ratio	0.0029 ± 0.004

Summary of study cohort characteristics including age, race, sex, and CD4 T-cell counts were appropriately shown as numbers (n), percentages (%), or mean ± SD.
AUDIT-C, Alcohol Use Disorders Identification Test criteria.

Age-dependent Alterations in Neuroactive TRP Metabolites in Older Adults Living With HIV

Disruptions in TRP metabolism have been linked to various neurological disorders.[15,47] In particular, neuroactive TRP metabolites, serotonin (5-hydroxytryptamine) and KYN, have been implicated in HIV-associated neurological disorders.[18,48,49] However, the effect of age on serotonin and KYN changes in HIV-infected population remains largely undetermined. Hence, we measured the plasma levels of serotonin and KYN in our older HIV-infected study population. The plasma levels of serotonin, KYN, and TRP were detected using LC-MS/MS method and documented in Table 1. A univariate linear regression analysis was performed between age and these metabolites to evaluate the age-associated changes in PLWH. A significant age-dependent decline was observed in plasma serotonin levels (Fig. 1A; $r^2 = -0.253$ and $P = 0.020$) in PLWH. By contrast, KYN was significantly increased in association with increasing age (Fig. 1B; $r^2 = 0.381$ and $P = 0.002$). In addition, the plasma KYN/TRP ratio, which is reflective of the TRP-degrading enzyme IDO activity, was increased in an age-dependent manner in

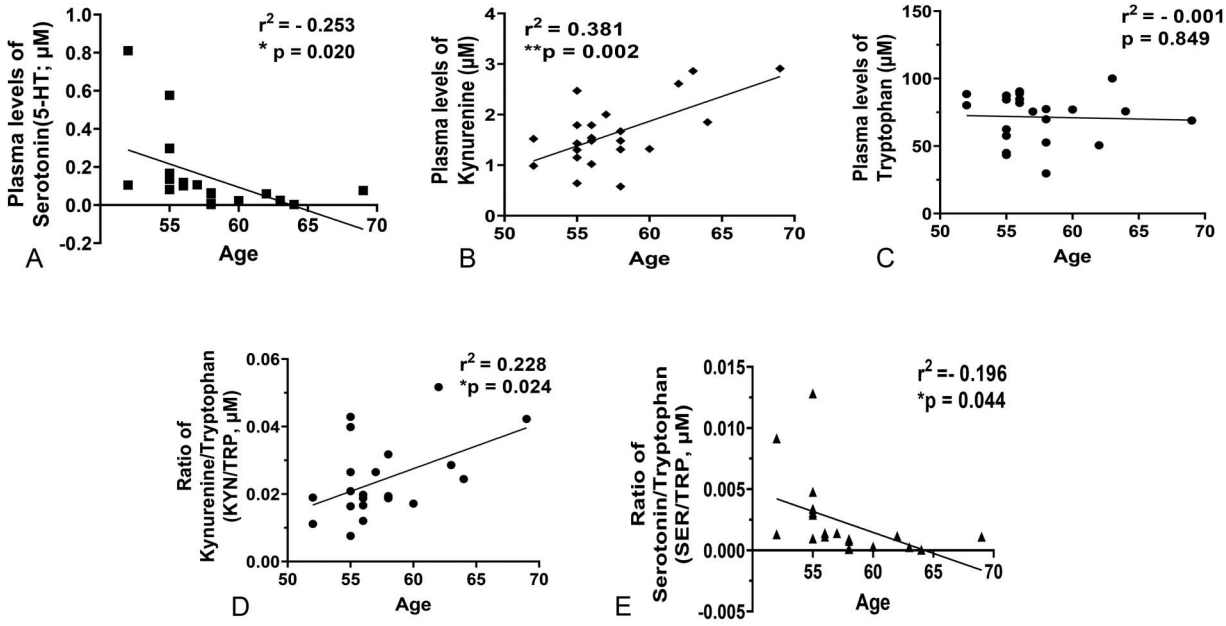

FIGURE 1. Age-dependent alteration in tryptophan metabolites in older PLWH: the metabolomic profiling of plasma serotonin, KYN, and tryptophan levels was performed using LC-MS/MS mass spectrometry. The graphs showing age-dependent correlation with plasma levels of (A) serotonin; (B) KYN; (C) TRP; (D) KYN/TRP ratio, and (E) SER/TRP ratio in older PLWH were plotted, and the linear regression coefficient (r^2) and statistical significance ($P < 0.05$) were denoted. SER, serotonin.

PLWH (Fig. 1D; $r^2 = 0.228$ and $P = 0.024$). Concurrently, plasma 5-HT to TRP (5-HT/TRP) ratio decreased with an increase in age (Fig. 1E; $r^2 = -0.195$ and $P = 0.044$), also potentially indicating an increase in IDO activity. In comparison, our data demonstrated no age-dependent change in plasma TRP levels (Fig. 1C; $r^2 = -0.001$ and $P = 0.849$). These data indicate that the net rate of TRP catabolism is not changing in an age-dependent fashion in our study population of PLWH. However, the proportion of TRP being catabolized along the 5-HT pathway is potentially shifted more towards the KYN pathway in an age-dependent fashion in PLWH. As a result, there is an increase in KYN and KYN/TRP ratio and a decrease in 5-HT and 5-HT/TRP ratio but no net change in the total pool of TRP. Taken together, the data show the development of age-dependent changes in the TRP metabolism and consequent imbalance of TRP-derived neuroactive metabolites in PLWH.

Gut Dysbiosis in Older Adults Living With HIV is Marked by Age-dependent Compositional and Functional Changes in the Microbiome

Because gut dysbiosis is known to affect TRP metabolism,[13,50] gut microbial dysbiosis was assessed in our study cohort of older PLWH. Using 16S rRNA gene sequencing strategy as detailed in the Methods, metagenomics analysis was performed on fecal samples. The data obtained provided detailed taxonomic information of the fecal microbial composition up to the bacterial genera level. A total of 321,491 high-quality mapped reads were obtained from the 22 older PLWH samples, with an average of 10,716 reads per sample, which were clustered into 2327 rarefied operational taxonomic units with 97% similarity. The taxonomy-based analysis demonstrated the presence of 8 phyla in the study population with Firmicutes

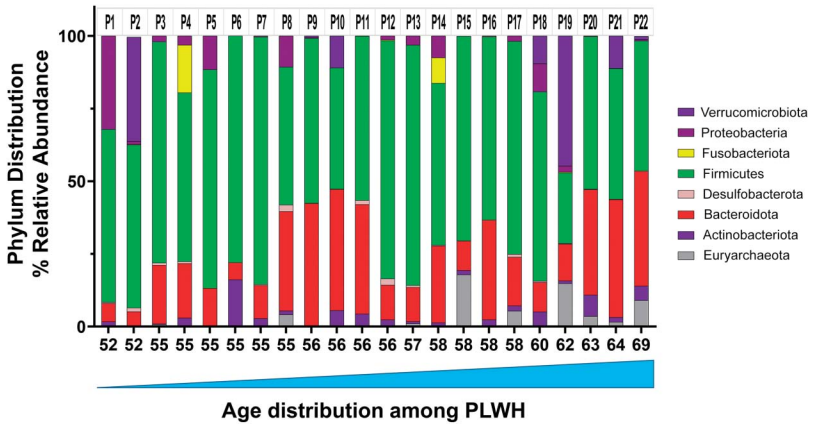

FIGURE 2. Phyla composition profile of older PLWH: a stack bar graph depicted the distribution of bacterial phyla based on percent relative abundance among 22 older PLWH. The taxonomic assignments of phyla were defined during 16S rRNA gene sequencing using the SILVA database. Each color represents the same phylum among all study participants.

(mean 61%), Bacteroidetes (mean 22%), and Verrucomicrobiota (mean 5%) as the predominant phyla and Proteobacteria (mean 4%), Actinobacteriota (mean 3%), Euryarchaeota (mean 3%), Fusobacteriota (mean 1%), Desulfobacterota (mean 1%) as the minor phyla (less than 5% relative abundance) (Fig. 2).

An examination of the 2 major bacterial phyla, Firmicutes and Bacteroidetes, revealed a significant age-dependent change in their relative abundance. Specifically, the univariate linear regression analysis showed that increasing age is significantly and negatively associated with Firmicutes (Fig. 3A; $r^2 = -0.197$ and $P = 0.038$) and positively associated with Bacteroidetes (Fig. 3B; $r^2 = 0.206$ and $P = 0.033$). Furthermore, the ratio of Firmicutes/Bacteroidetes (F/B), a well-known marker of gut microbial dysbiosis, showed a significant age-dependent decline (Fig. 3C; $r^2 = -0.253$ and $P = 0.016$), indicating temporal shifts in gut microbial composition in older adults with HIV infection. In addition, racial differences in the study were noted with White study participants showing an age-dependent decrease in F/B ratio ($r^2 = -0.453$ and $P = 0.016$). It is interesting to note that although the F/B ratio changed, there was no significant age-dependent change in species richness and evenness, as denoted by the alpha diversity measures—Shannon index

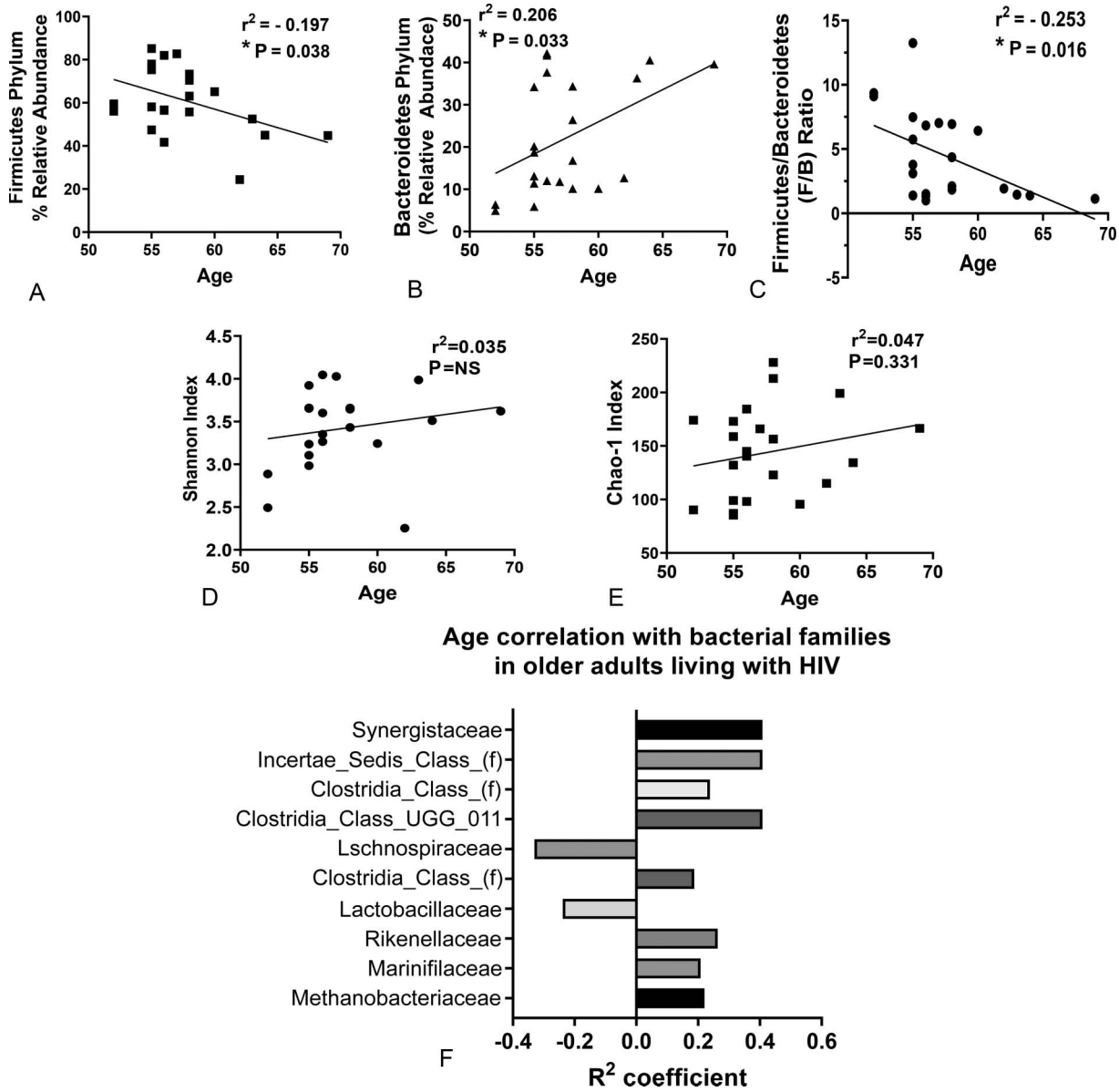

FIGURE 3. Aging in PLWH is marked by gut dysbiosis: percentage relative abundance of Firmicutes and Bacteroidetes phyla was calculated from 16S rRNA gene sequencing. Linear regression graphs documenting the correlation between age and (A) Firmicutes phylum (B) Bacteroidetes phylum; (C) F/B ratio and microbial diversity indicators (D) Simpson index; and (E) Chao-1 index were shown. The regression coefficient (r^2) and statistical significance ($P < 0.05$) were denoted on each graph. F, Summary bar graph showing significant ($P < 0.05$) age correlation with bacterial families was plated using regression coefficient.

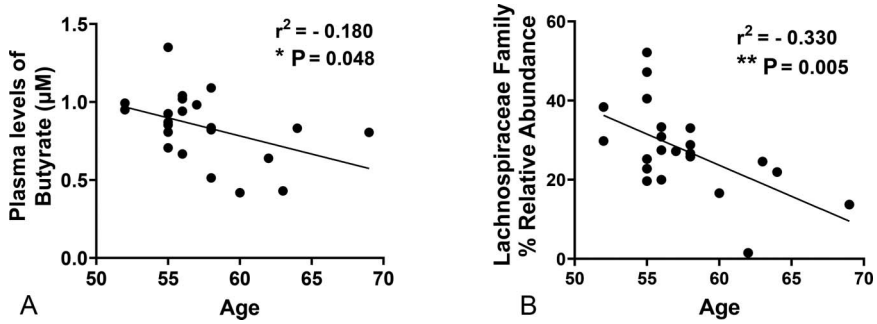

FIGURE 4. Age-associated loss of butyrate levels and butyrate-producing family in older PLWH: Linear regression analysis was used to determined age-dependent changes in (A) plasma butyrate levels and (B) percentage relative abundance of Lachnospiraceae family in older PLWH.

and Chao-1 index (Figure 3D; $r^2 = 0.035$ and $P = 0.401$ and Fig. 3E; $r^2 = 0.047$ and $P = 0.331$).

Furthermore, characterization of age-dependent microbial changes at the bacterial family level revealed that there is (1) a decrease in Lactobacillaceae and Lachnospiraceae families belonging to the Firmicutes phylum and (2) increase in Marinifilaceae and Rikenellaceae families from Bacteroidetes phylum (Fig. 3F). In addition, the Methanobacteriaceae family from the Euryarchaeota phylum, 3 unknown families belonging to Firmicutes, and the Synergistaceae family from Synergistota phylum were also increased along with age in older PLWH (Fig. 3F).

After the determination of the age-dependent compositional changes in the microbial communities, the functional consequence of dysbiosis was evaluated. Because bacterially derived short-chain fatty acids, particularly butyrate, can significantly affect host TRP metabolism,[51] plasma butyrate levels were assessed by LC-MS/MS. The data showed that butyrate levels significantly decreased in the PLWH population with an increment in age (Fig. 4A; $r^2 = -0.180$ and $P = 0.048$). In additionally, in accordance with the loss of plasma butyrate levels, a significant age-dependent decrease in the largest butyrate-producing bacterial family, Lachnospiraceace (Fig. 4B; $r^2 = -0.330$ and $P = 0.005$), and butyrate synthesis–promoting family—Lactobacillaceae ($r^2 = -0.239$ and $P = 0.021$) was observed.

Microbial Compositional and Functional Dysbiosis Correlates With Age-Dependent Alterations in Neuroactive TRP Metabolites, 5-HT, and KYN in Older Adults Living With HIV

In relevance to the known effects of microbial-derived butyrate on the generation of TRP metabolites, Spearman correlation analysis was performed to examine the relationship between the levels of butyrate and TRP metabolites (Table 2) in older PLWH. The data demonstrated that the decrease in plasma butyrate levels significantly correlates with decreasing serotonin levels and conversely with increasing KYN/TRP, further suggesting that functional deficits of metabolites are linked to gut microbial dysbiosis in older PLWH.

Next, we examined the correlation of the butyrate-producing bacterial genera with the neuroactive TRP metabolites serotonin and KYN (Table 3). Linear regression analysis of the acquired metagenomics sequencing data and serotonin showed that *Lachnoclostridium* and *Lachnospiraceae_FCS020_group* genera belonging to the Lachnospiraceae family, *Holdemanella* sp. and *Turicibacter* sp. belonging to the Erysipelatoclostridiaceae family were directly associated with serotonin levels.[52–56] In additionally, *Lagilactobacillus* sp. and *Lacticaseibacillus* sp. from the Lactobacillaceae family known to support the growth of butyrate producers[57,58] were also positively associated with serotonin levels (Table 3).

Of interest, *Tyzzerella* sp. belonging to the Lachnospiraceae family that plays a role in regulating serotonin release through tryptamine production (one of the TRP metabolites)[13] was found to be positively correlated with serotonin in these older PLWH study cohort (Table 3). Conversely, a decrease in *Allisonella* sp., butyrate-producing genera belonging to the Veillonellaceae family, correlated with an increase in KYN in older PLWH[59] (Table 3).

In addition, *Staphylococcus* sp. and *Pyramidobacter* sp. that were found in higher abundance in neurological disorders[60,61] showed a direct correlation with KYN. Furthermore, the *Ruminococcus_gnavus_group* genera that convert TRP to tryptamine using enzyme TRP decarboxylase were negatively correlated with KYN[13,62] (Table 3).

Overall, these data implicate that age-associated decrease in butyrate-producing bacteria (compositional change) and consequent decline in butyrate levels (functional change) influence age-dependent decrease in 5-HT and increase in KYN levels in older adults living with HIV.

DISCUSSION

In the post-Combination Antiretroviral Therapy era, HIV+ adults are surviving and reaching advanced age with near-normal life expectancy. Yet, they live with a chronic viral infection, which may remain latent, but which continues to affect organ systems such as the gut and brain. There is

TABLE 2. Spearman Correlation Analysis

	Serotonin (5-HT)	Kynurenine	KYN/TRP
Butyrate	r = 0.421	r = −0.295	r = −0.522
	$P = 0.05$	NS	$P = 0.013$

r: Spearman coefficient; statistically significant at $P \leq 0.05$.
NS, not significant.

TABLE 3. Gut Microbial Genera Associated With Alterations in Tryptophan Metabolites 5-HT and Kynurenine in Older PLWH

	Taxonomic Classification			Regression Coefficient	Significance
Metabolite	**Phylum**	**Family**	**Genera**	**r^2**	***P***
Serotonin (5-HT)	Firmicutes	Lachnospiraceae	*Lachnoclostridium* sp.	0.4142	0.0016
			Lachnospiraceae_FCS020_group sp.	0.2535	0.02
			Tyzzerella sp.	0.5389	0.0002
		Erysipelatoclostridiaceae	*Holdemanella* sp.	0.4045	0.0019
			Turicibacter sp.	0.2729	0.0151
		Lactobacillaceae	*Lagilactobacillus* sp.	0.5741	<0.0001
			Lacticaseibacillus sp.	0.2349	0.026
Kynurenine	Firmicutes	Veillonellaceae	*Allisonella* sp.	−0.3703	0.0027
		Lachnospiraceae	*Ruminococcus_gnavus_group* sp.	−0.1843	0.0462
		Staphylococcaceae	*Staphylococcus* sp.	0.2923	0.0094
	Synergistota	Synergistaceae	*Pyramidobacter* sp.	0.2025	0.0356

Linear regression analysis was performed, and regression coefficients and statistical significance (*P*-value) were shown for each bacterial genus.

Summary table documenting direct (r2) and inverse (- r2) relationship between gut bacterial genera with serotonin and kynurenine. Linear regression analysis was performed and regression coefficients and statistical significance (*p*-value) were shown for each bacterial genera.

evidence of greater than expected cognitive deficits in older HIV+ adults and premature decline in learning memory, suggesting premature cognitive aging and/or neurodegeneration. In this regard, dysregulation of TRP metabolism is an important clinical feature of both aging and HIV infection, contributing to the development of neurodegenerative diseases and loss of neurological function infection.[16–19] Preclinical and clinical studies have demonstrated that compositional changes in the gut microbiome can influence cognitive function.[63] Moreover, there is gathering evidence for the microbial regulation of TRP metabolism and the generation of neuroactive molecules such as serotonin and KYN that can affect neurocognitive outcomes.[31–35] Hence, this pilot study examined the potential interactive effects of HIV infection and aging in the context of compositional and functional changes in the gut microbiome and its correlation with alterations in TRP metabolism.

Important findings regarding the changes in gut microbial composition and function in this pilot study population of older PLWH were (1) the age-dependent decline in Lachnospiraceae and Lactobacillus families that host several butyrate-producing genera and (2) consequent loss of the butyrogenic potential demonstrated by an incremental loss of plasma butyrate levels with increasing age. Of note, low abundance of butyrate-producing bacteria has also been observed in HIV-infected individuals who were relatively younger with an average age of 32.5 years.[64]

Significantly, along with a decrease in butyrate levels, there was a concomitant age-dependent decline in 5-HT levels. In this regard, it is noteworthy that gut microbiome-derived butyrate affects TRP metabolism and increases the production of the neurotransmitter 5-HT. It has been demonstrated that gut bacteria–derived butyrate induces mRNA expression of TRP hydroxylase—the rate-limiting enzyme in 5-HT biosynthesis in intestinal ECs, thereby increasing 5-HT production[51]; moreover, butyrate also stimulates 5-HT release from ECs.[65] Taken together, our data suggest that the change in gut microbial composition (dysbiosis) and consequent loss of its butyrogenic potential could be a significant mechanism involved in the age-dependent decline in the 5-HT biosynthetic TRP-hydroxylation pathway and loss of 5-HT levels in older PLWH.

Notably, in contrast to the decrease in plasma 5-HT levels, there was an age-dependent increase in the KYN levels and KYN: TRP (KYN/TRP) ratio in the older PLWH. Our findings further extend the earlier studies that have independently examined the effects of either HIV infection or aging and have observed a link between gut dysbiosis and altered TRP metabolism and an increase in KYN/TRP ratio.[28,35,66,67] The increase in metabolic intermediates such as KYN implicates a shift of the TRP metabolism more towards the TRP oxidation pathway involving an increase in the IDO enzyme activity.[26] Moreover, KYN/TRP ratio has been demonstrated to be a reliable marker of IDO activity that regulates the initial and rate-limiting step in TRP oxidation and its conversion to KYN.[68,69] Accordingly, our data suggest that in the older PLWH, there is an age-related increase in the IDO activity and a resultant shift to the TRP oxidation pathway, increasing KYN levels and KYN: TRP (KYN/TRP) ratio in an age-dependent manner. Of importance, because IDO activity is downregulated by butyrate produced by the commensal bacteria,[54] our data indicate that the age-associated functional changes in the gut microbiome and loss of butyrogenic potential is directly or indirectly linked to the enhancement of the oxidative conversion of TRP to KYN in older PLWH. Furthermore, HIV infection is often associated with increase in circulating levels of inflammatory cytokines such as interleukin-6 and interferon-gamma.[70,71] Because these inflammatory cytokines are known to stimulate IDO activity, which converts TRP metabolism toward KYN pathway, future studies investigating the relationship between age-associated increase in KYN/TRP ratio and chronic inflammation are warranted. Observational data suggest that KYN/TRP ratio is a marker for inflammaging and is involved in the onset of age-related diseases. In this regard, studies in elderly individuals have demonstrated an increased KYN/

TRP ratio to be linked with increased risk of cardiovascular disease,[72,73] reduced cognitive performance,[17] increased frailty,[74] and mortality.[72,75] Hence, the current findings suggest that the pathogenic role of age-dependent gut microbial dysbiosis, leading to loss of butyrogenic potential and alterations in TRP metabolism, could be explored in clinically relevant age-related diseases.

We acknowledge that our study findings have certain limitations. In particular, the study was a pilot cross-sectional study conducted to establish a conceptual framework to determine metagenomics and metabolomics parameters that would be clinically relevant for the examination of the older PLWH population. Further studies using a larger study cohort will be required to validate the initial findings made in this study. Because our study population predominantly consisted of men, a similar investigation in women will provide better insight into the age-associated changes in TRP metabolism in PLWH. In addition, only univariate analyses have been performed without the adjustment for covariates such as sex, alcohol use, and CD4/CD8 T-cell status. However, the regression coefficients are sufficiently high to support the suggested outcomes.

The findings from this pilot study have begun to address the significant aspects of the age-dependent compositional and functional changes in the gut microbiome that are relevant for the regulation of serotonin synthesis and the control of the KYN pathway and the development of neurocognitive impairment in older PLWH. Overall, the data suggest that the progressive loss of butyrogenic potential is a significant pathogenic feature of age-associated gut microbial dysbiosis that adversely affects TRP metabolism. Of importance, the data also provide a clinical rationale for targeting the gut dysbiosis–mediated loss of butyrogenic potential to favorably modulate the TRP metabolism in older PLWH.

REFERENCES

1. Sabin CA, Reiss P. Epidemiology of ageing with HIV: what can we learn from cohorts? *AIDS*. 2017;31(suppl 2):S121–S128.
2. Schouten J, Wit FW, Stolte IG, et al. Cross-sectional comparison of the prevalence of age- associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV cohort study. *Clin Infect Dis*. 2014;59:1787–1797.
3. May M, Gompels M, Delpech V, et al. Impact of late diagnosis and treatment on life expectancy in people with HIV-1: UK Collaborative HIV Cohort (UK CHIC) Study. *BMJ*. 2011;343:d6016.
4. Meir-Shafrir K, Pollack S. Accelerated aging in HIV patients. *Rambam Maimonides Med J*. 2012;3:e0025.
5. Kent SJ, Flexner C. Ageing in patients with chronic HIV infection: impact of hypercoagulation. *AIDS Res Ther*. 2018;15:22.
6. Horvath S, Levine AJ. HIV-1 infection accelerates age according to the epigenetic clock. *J Infect Dis*. 2015;212:1563–1573.
7. Kooij KW, Wit FW, Schouten J, et al. HIV infection is independently associated with frailty in middle-aged HIV type 1-infected individuals compared with similar but uninfected controls. *AIDS*. 2016;30:241–250.
8. Guaraldi G, Orlando G, Zona S, et al. Premature age-related comorbidities among HIV-infected persons compared with the general population. *Clin Infect Dis*. 2011;53:1120–1126.
9. Aung HL, Aghvinian M, Gouse H, et al. Is there any evidence of premature, accentuated and accelerated aging effects on neurocognition in people living with HIV? A systematic Review. *AIDS Behav*. 2021;25: 917–960.
10. Kamkwalala A, Newhouse P. Mechanisms of cognitive aging in the HIV-positive adult. *Curr Behav Neurosci Rep*. 2017;4:188–197.
11. Cohen RA, Seider TR, Navia B. HIV effects on age-associated neurocognitive dysfunction: premature cognitive aging or neurodegenerative disease? *Alzheimers Res Ther*. 2015;7:37.
12. Becker JT, Lopez OL, Dew MA, et al. Prevalence of cognitive disorders differs as a function of age in HIV virus infection. *AIDS*. 2004;18(suppl 1):S11–S18.
13. Kaur H, Bose C, Mande SS. Tryptophan metabolism by gut microbiome and gut-brain-axis: an in silico analysis. *Front Neurosci*. 2019;13:1365.
14. O'Mahony SM, Clarke G, Borre YE, et al. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res*. 2015;277:32–48.
15. Roth W, Zadeh K, Vekariya R, et al. Tryptophan metabolism and gut-brain homeostasis. *Int J Mol Sci*. 2021;22:2973.
16. Kim BJ, Lee SH, Koh JM. Clinical insights into the kynurenine pathway in age-related diseases. *Exp Gerontol*. 2020;130:110793.
17. Solvang SH, Nordrehaug JE, Tell GS, et al. The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study. *Brain Behav Immun*. 2019;75:155–162.
18. Keegan MR, Chittiprol S, Letendre SL, et al. Tryptophan metabolism and its relationship with depression and cognitive impairment among HIV-infected individuals. *Int J Tryptophan Res*. 2016;9:79–88.
19. Davies NW, Guillemin G, Brew BJ. Tryptophan, neurodegeneration and HIV-associated neurocognitive disorder. *Int J Tryptophan Res*. 2010;3:121–140.
20. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. *Annu Rev Med*. 2009;60:355–366.
21. Jonnakuty C, Gragnoli C. What do we know about serotonin? *J Cell Physiol*. 2008;217:301–306.
22. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 2015;161:264–276.
23. Deza-Araujo YI, Baez-Lugo S, Vuilleumier P, et al. Whole blood serotonin levels in healthy elderly are negatively associated with the functional activity of emotion-related brain regions. *Biol Psychol*. 2021; 160:108051.
24. Launay JM, Copel L, Callebert J, et al. Decreased whole blood 5-hydroxytryptamine (serotonin) in AIDS patients. *J Acquir Immune Defic Syndr*. 1988;1:324–325.
25. Tan L, Yu JT, Tan L. The kynurenine pathway in neurodegenerative diseases: mechanistic and therapeutic considerations. *J Neurol Sci*. 2012; 323:1–8.
26. Clarke G, McKernan DP, Gaszner G, et al. A distinct profile of tryptophan metabolism along the kynurenine pathway downstream of toll-like receptor activation in irritable bowel syndrome. *Front Pharmacol*. 2012;3:90.
27. Hajsl M, Hlavackova A, Broulikova K, et al. Tryptophan metabolism, inflammation, and oxidative stress in patients with neurovascular disease. *Metabolites*. 2020;10.
28. Sorgdrager FJH, Naude PJW, Kema IP, et al. Tryptophan metabolism in inflammaging: from biomarker to therapeutic target. *Front Immunol*. 2019;10:2565.
29. Gostner JM, Becker K, Kurz K, et al. Disturbed amino acid metabolism in HIV: association with neuropsychiatric symptoms. *Front Psychiatry*. 2015;6:97.
30. Fuchs D, Moller AA, Reibnegger G, et al. Increased endogenous interferon-gamma and neopterin correlate with increased degradation of tryptophan in human immunodeficiency virus type 1 infection. *Immunol Lett*. 1991;28:207–211.
31. Szoke H, Kovacs Z, Bokkon I, et al. Gut dysbiosis and serotonin: intestinal 5-HT as a ubiquitous membrane permeability regulator in host tissues, organs, and the brain. *Rev Neurosci*. 2020;31:415–425.
32. Kennedy PJ, Cryan JF, Dinan TG, et al. Kynurenine pathway metabolism and the microbiota- gut-brain axis. *Neuropharmacology*. 2017;112(pt B): 399–412.
33. Kim M, Benayoun BA. The microbiome: an emerging key player in aging and longevity. *Transl Med Aging*. 2020;4:103–116.
34. Ruiz-Ruiz S, Sanchez-Carrillo S, Ciordia S, et al. Functional microbiome deficits associated with ageing: chronological age threshold. *Aging Cell*. 2020;19:e13063.
35. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med*. 2013;5:193ra191.

36. Han J, Lin K, Sequeira C, et al. An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography-tandem mass spectrometry. *Anal Chim Acta*. 2015;854:86–94.
37. Zhang L, Zheng J, Ahmed R, et al. A high-performing plasma metabolite panel for early-stage lung cancer detection. *Cancers (Basel).* 2020;12: 622.
38. Human Microbiome Project Consortium. A framework for human microbiome research. *Nature*. 2012;486:215–221.
39. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207–214.
40. Thompson LR, Sanders JG, McDonald D, et al. A communal catalogue reveals earth's multiscale microbial diversity. *Nature*. 2017;551: 457–463.
41. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 2012;6:1621–1624.
42. Amir A, McDonald D, Navas-Molina JA, et al. Deblur rapidly resolves single-nucleotide community sequence patterns. *mSystems.* 2017;2: e00191–e00216.
43. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 2013;41:D590–D596.
44. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010;26:2460–2461.
45. Katoh K, Toh H. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics.* 2010;26:1899–1900.
46. Price MN, Dehal PS, Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS One.* 2010;5:e9490.
47. Strasser B, Gostner JM, Fuchs D. Mood, food, and cognition: role of tryptophan and serotonin. *Curr Opin Clin Nutr Metab Care*. 2016;19: 55–61.
48. Qi Q, Hua S, Clish CB, et al. Plasma tryptophan-kynurenine metabolites are altered in human immunodeficiency virus infection and associated with progression of carotid artery atherosclerosis. *Clin Infect Dis.* 2018; 67:235–242.
49. Huengsberg M, Winer JB, Gompels M, et al. Serum kynurenine-to-tryptophan ratio increases with progressive disease in HIV-infected patients. *Clin Chem.* 1998;44:858–862.
50. Gao K, Mu CL, Farzi A, et al. Tryptophan metabolism: a link between the gut microbiota and brain. *Adv Nutr.* 2020;11:709–723.
51. Reigstad CS, Salmonson CE, Rainey JF III, et al. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* 2015;29:1395–1403.
52. Kort R, Schlosser J, Vazquez AR, et al. Model selection reveals the butyrate-producing gut bacterium coprococcus eutactus as predictor for language development in 3-year-old rural Ugandan children. *Front Microbiol.* 2021;12:681485.
53. Gutierrez N, Garrido D. Species deletions from microbiome consortia reveal key metabolic interactions between gut microbes. *mSystems.* 2019: 4:e00185–e00219.
54. Martin-Gallausiaux C, Larraufie P, Jarry A, et al. Butyrate produced by commensal bacteria down-regulates indolamine 2,3-dioxygenase 1 (Ido-1) expression via a dual mechanism in human intestinal epithelial cells. *Front Immunol.* 2018;9:2838.
55. Vital M, Karch A, Pieper DH. Colonic butyrate-producing communities in humans: an overview using omics data. *mSystems.* 2017;2:e00130–17.
56. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol.* 2017;19:29–41.
57. Nagpal R, Wang S, Ahmadi S, et al. Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. *Sci Rep.* 2018;8:12649.
58. Pessione E. Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Front Cell Infect Microbiol.* 2012;2:86.
59. Hernandez-Sanabria E, Heiremans E, Calatayud Arroyo M, et al. Short-term supplementation of celecoxib-shifted butyrate production on a simulated model of the gut microbial ecosystem and ameliorated in vitro inflammation. *NPJ Biofilms Microbiomes.* 2020;6:9.
60. Ren T, Gao Y, Qiu Y, et al. Gut microbiota altered in mild cognitive impairment compared with normal cognition in sporadic Parkinson's disease. *Front Neurol.* 2020;11:137.
61. Hassel B, Dahlberg D, Mariussen E, et al. Brain infection with Staphylococcus aureus leads to high extracellular levels of glutamate, aspartate, gamma-aminobutyric acid, and zinc. *J Neurosci Res.* 2014;92: 1792–1800.
62. Williams BB, Van Benschoten AH, Cimermancic P, et al. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host Microbe.* 2014;16:495–503.
63. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe.* 2018;23:716–724.
64. Dillon SM, Kibbie J, Lee EJ, et al. Low abundance of colonic butyrate-producing bacteria in HIV infection is associated with microbial translocation and immune activation. *AIDS*. 2017;31:511–521.
65. Fukumoto S, Tatewaki M, Yamada T, et al. Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am J Physiol Regul Integr Comp Physiol.* 2003;284:R1269–R1276.
66. Gao J, Xu K, Liu H, et al. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. *Front Cell Infect Microbiol.* 2018;8:13.
67. Rampelli S, Candela M, Turroni S, et al. Functional metagenomic profiling of intestinal microbiome in extreme ageing. *Aging*. 2013;5: 902–912.
68. Raison CL, Dantzer R, Kelley KW, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry*. 2010;15:393–403.
69. Schrocksnadel K, Wirleitner B, Winkler C, et al. Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta*. 2006;364: 82–90.
70. Zevin AS, McKinnon L, Burgener A, et al. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. *Curr Opin HIV AIDS.* 2016;11:182–190.
71. Aberg JA. Aging, inflammation, and HIV infection. *Top Antivir Med.* 2012;20:101–105.
72. Zuo H, Ueland PM, Ulvik A, et al. Plasma biomarkers of inflammation, the kynurenine pathway, and risks of all-cause, cancer, and cardiovascular disease mortality: the hordaland health study. *Am J Epidemiol.* 2016;183:249–258.
73. Sulo G, Vollset SE, Nygard O, et al. Neopterin and kynurenine-tryptophan ratio as predictors of coronary events in older adults, the Hordaland Health Study. *Int J Cardiol.* 2013;168:1435–1440.
74. Valdiglesias V, Marcos-Perez D, Lorenzi M, et al. Immunological alterations in frail older adults: a cross sectional study. *Exp Gerontol.* 2018;112:119–126.
75. Ramos-Chavez LA, Roldan-Roldan G, Garcia-Juarez B, et al. Low serum tryptophan levels as an indicator of global cognitive performance in nondemented women over 50 years of age. *Oxid Med Cell Longev.* 2018; 2018:8604718.