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Gut microbiota and cognitive function among women living with HIV

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Abstract

Background: Altered gut microbiota has been associated with cognitive dysfunction and Alzheimer's disease, but little is known among people living with HIV.

Objective: To examine associations between gut microbiota and cognitive impairment among women with or without HIV.

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Conflict of Interest/Disclosure Statement

The authors have no conflict of interest to report.

Methods: This is a cross-sectional study of 446 women (302 HIV+) who had completed a neuropsychological test battery and stool sample collected within 1 year. Gut microbiota composition was quantified using 16SV4 rRNA gene sequencing and microbial functional pathways were predicted using PICRUSt. Cognitive domains included attention, executive function, learning, memory, fluency, processing speed, and motor function. Cognitive impairment was defined as two or more domains with T scores<1 SD below mean. ANCOM-II was used to identify taxa and functional pathways associated with cognitive impairment, and the associations were further examined by multivariable logistic regression.

Results: In overall sample, adjusting for multiple covariates including HIV status, we found that higher abundance of *Methanobrevibacter*, *Odoribacter*, *Pyramidobacter*, *Eubacterium*, *Ruminococcus*, and *Gemmiger*, and lower abundance of *Veillonella* were associated with cognitive impairment. The associations between these taxa and cognitive impairment were more profound in HIV+ women compared to HIV− women. Most associations with bacterial taxa were observed for learning and memory. We found accompanying microbial functional differences associated with cognitive impairment, including twelve enriched pathways and three depleted pathways.

Conclusion: In women with or without HIV infection, this study identified multiple altered gut bacterial taxa and functional pathways associated with cognitive impairment, supporting the potential role of gut microbiota in cognitive dysfunction and Alzheimer's disease.

Keywords

HIV; Cognitive Impairment; Gut Microbiome; Alzheimer’s Disease; Human; Women

Introduction

With the availability of combination antiretroviral therapy (cART), the overall mortality of people living with Human Immunodeficiency Virus (HIV) has decreased greatly and more attention has shifted to aging related diseases among this population. When including asymptomatic disease, 31-47% of people living with HIV (PLWH) are estimated to have cognitive impairment. [1, 2] Among PLWH, the majority of individuals with cognitive impairment now demonstrates mild forms of disease. [1] A previous study in the Women’s Interagency Health Study (WIHS) found that HIV infection status was associated with significant cognitive deficits in processing speed, attention, verbal learning and delayed memory.[3] In addition, older PLWH are also at risk for Alzheimer's disease which might be due to compounding effects of HIV and aging.[4] The potential causes of cognitive impairment in PLWH have been suggested, including incomplete viral suppression in the central nervous system (CNS), neural injury due to viral proteins and inflammatory responses, neurotoxicity of ART, metabolic disorders and increased amyloid-β deposition in the brain. [5] However, the actual mechanism is still not well understood.

The gut-brain axis involves bi-directional communication between gut microbiota (GMB) and the CNS through neuronal, endocrine, and immune-mediated processes.[6-8] Studies have shown that GMB may play a part in the development of neurodegenerative diseases.[9, 10] It has been reported that, as compared with people with normal cognitive function, those with mild cognitive impairment and Alzheimer’s disease

showed altered GMB profiles, including reduced *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiaceae*, *Mogibacteriaceae*, *Turicibacteaceae* and *Peptostreptococcaceae* families, *Lachnospira*, *Ruminiclostridium*, *Dialister*, *Clostridium* and *Bifidobacterium* genera, and enriched Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Rikenellaceae, *Alistipes*, *Prevotella*, *Odoribacter*, and *Barnesiella*. [10-14] Microbial dysbiosis, such as increased abundance of pro-inflammatory bacteria and decreased abundance of anti-inflammatory bacteria, may influence the immune system and lead to local and systemic inflammation. [15] Translocation of bacteria and increased permeability of the gut epithelial barrier and blood-brain barrier result in neuroinflammatory response in the brain.[16] Additionally, microbial-produced bioactive metabolites, such as short-chain fatty acids (SCFA), serotonin, kynurenine and amyloids, play essential roles in neurotransmission and neuromodulation. [15, 16]

While prior evidence provides a basis for linking the gut-brain axis with cognitive disorders, the sample sizes of existing studies are relatively small (N<200) and most studies included participants in the hospital setting. Moreover, to the best of our knowledge, no study has investigated the relationship between GMB and cognitive impairment in the context of HIV infection, although PLWH are at risk for gut dysbiosis.[17] Thus, in this study, we examined associations between gut bacterial features (overall diversity, individual bacterial taxa and bacterial functional pathways) and cognitive impairment among 446 women with or without HIV from a community-based HIV cohort, the WIHS. Moreover, we hypothesized that some of the associations might be stronger in PLWH due to gut barrier dysfunction, which may enhance microbial translocation,[18] compared to those without HIV. Thus, we also explored potential effect modification by HIV serostatus on the association between GMB and cognitive impairment.

Materials and Methods

Study population

The WIHS is a prospective cohort study of women with and at risk for HIV since 1994, now part of the MACS WIHS Combined Cohort Study (MWCCS). [19-21] Participants were recruited from 10 cities in the United States were followed up every 6 months to collect biospecimens, medical history, medication use, health-related behaviors and anthropometry. To ensure comparability with HIV+ women, HIV− women who engaged in high risk behaviors for HIV were recruited.[19] Since 2009 to 2019, participants were administered a comprehensive neuropsychological testing every 2 years.[3] From 2016 to 2019, the Bronx, Brooklyn, and Chicago WIHS sites collected stool samples from participants.[22] Written informed consent was obtained from participants. The study was approved by institutional review boards at each site.

We included 466 WIHS participants who provided stool samples, which were obtained within about 1 year either before or after completion of a neuropsychological test battery. After excluding 11 samples with low sequencing depth (<2000 sequence reads per sample) and 9 samples with missing data >3 domains of a neuropsychological test, a total of 446 participants were in the study sample, 302 of whom were HIV+ women.

Cognitive function.—Participants completed a neuropsychological test battery assessing the following domains: learning (Hopkins Verbal Learning Test-Revised [HVLT-R]-total learning across trials 1 to 3); memory (HVLT-R-delay free recall); psychomotor speed (Symbol Digit Modalities Test, Stroop-Trial 2); attention/working (Letter Number Sequencing); motor function (Grooved Pegboard dominant and non-dominant hands); verbal fluency (letter and semantic); and executive function (Trail Making Test Part B and Stroop Test Interference Trial). [23] Timed outcomes were log transformed to normalize distributions and reverse scored, so higher equated to better performance. As previously described,[2, 3, 23] demographically-adjusted T scores were then derived for each cognitive domain using data from the HIV− women. Age, years of education, Wide Range Abilities Test (WRAT)-3 Reading Recognition subtest score, race/ethnicity, and number of prior neuropsychological test completions were included in the regression equations,[3] and domain-specific T-scores were then created. Most of the participants (94%) included in the study sample had scores available in all 7 domains. Impairment on each domain was defined as T-scores < 1 standard deviation (SD) below the mean of the HIV− women. [2, 23] If participants had two or more domains with impairment, they were considered to have global cognitive impairment. [2] The primary outcome of interest is global cognitive impairment. The secondary outcomes are impairment in each domain. In addition, we also created a global performance score by averaging domain-specific T-scores as a continuous global cognitive measure.

Stool sample collection and microbiome measurement.—Stool samples were collected using a home-based self-collection kit containing RNAlater prepared in the laboratory of Dr. Robert D Burk at Albert Einstein College of Medicine. [22] In brief, stool sample was self-collected and placed in a supplied container including a stabilizer (RNAlater) and 0.5 mm diameter glass beads and instructed to shake the tube in order to mix the stool and the preservative which stabilizes DNA and RNA.[24] After collection, stool samples were stored at room temperature and mailed back to the lab through USPS. The lab froze the samples immediately at −80 °C upon receipt. As described previously, 16S rRNA V4 gene region amplification was performed on DNA extracted from stool samples using a bead-beating procedure by the MiSeq platform (Illumina, San Diego, CA) at Albert Einstein College of Medicine Sequencing Core.[25]

Bioinformatic analysis

Microbiome bioinformatics analyses were performed using the Quantitative Insights Into Microbial Ecology (QIIME2) software package (2019.10) with the Deblur pipeline.[26] The α-diversity indices (Shannon index and observed amplicon sequencing variant (ASV)) and β- diversity Jensen Shannon Divergence were calculated using QIIME2 and R phyloseq package after rarefication at 40 different sequencing depths (from 20 to 35,000 sequence reads per sample).[27] The functional potential of the GMB was imputed by PICRUSt (to calculate estimated relative abundances of KEGG ortholog groups).[28] We excluded samples with sequencing depths <2000 sequence reads per sample after the Deblur workflow.[25] After this exclusion, the lowest sequencing depth was 2507 in the study sample. Detailed quality control assessment was previously reported. [25]

Covariates

Data on age, race, education, annual income, health behaviors (recreational drug use and smoking status) and medication use, and blood samples were collected using standardized protocols at semiannual core study visits.[29] Recreational drugs include marijuana, crack, cocaine, heroin or injection drug use. Smoking status was assessed by current, ever or never smoking. ART include protease inhibitors, nucleoside and non-nucleoside reverse transcriptase inhibitors, and information on participant use of each type of drugs in the past six months was collected. HIV serostatus was ascertained using the enzyme linked immunosorbent assay method and confirmed by Western blot. Hepatitis C virus (HCV) infection was based on a serological test for antibodies or a nucleic acid test for viral RNA. Other HIV-related characteristics include cluster of differentiation 4^+ ($CD4^+$) cell count, HIV RNA and ART use. Undetectable HIV-1 viral load was defined as ⩽20 copies/mL.

Statistical analysis

Characteristics of HIV+ and HIV− women were compared using t-test for continuous variables and chi-squared test for categorical variables. Microbial α-diversity indices Shannon index and number of observed ASVs were compared by cognitive impairment using Wilcoxon rank-sum test. Permutational multivariate ANOVA (PERMANOVA) and principal-coordinate analysis (PCoA) were used to examine the differences in microbial β-diversity by cognitive impairment. For taxa and functional pathways analysis, we conducted the Analysis of Composition of Microbiomes (ANCOM-II), given its good control of false discovery rate (FDR), to identify candidates associated with global cognitive impairment.[30] We kept taxa that were present in at least 25% of samples with mean relative abundance > 0.01%. All models adjusted for age, race, education (below high school, high school and above high school), poverty (annual income ⩽$12000), recreational drug use, HIV status, HCV infection, site, antibiotic use, smoking (never, former and current), HIV viral load and ART use (only among HIV+ women). We included HIV viral load (detectable vs. undetectable) and ART use as categorical variables in which an additional level was created for HIV− women. These covariates were considered as potential confounders based on our previous analyses on GMB or cognitive function in the WIHS.[23, 31] We conducted ANCOM-II using raw count data and FDR threshold at 0.10, at multiple taxonomic levels including phylum (n=14), class (n=25), order (n=41), family (n=74), genus (n=168), and species (n=142), and for functional pathways (n=353). The analysis excluded unknown taxa at these taxonomic levels. An ANCOM-II detection level ≥ 0.60 indicates that the ratios of a taxon to at least 60% of other taxa were significantly different by cognitive impairment status, adjusting for multiple testing (FDR $q<0.10$). We examined multiple taxonomic levels to see whether the results were consistent within a taxonomic lineage, and we controlled for multiple testing by using FDR at each taxonomic level.

We used multivariable logistic regression to estimate the odds ratios (OR) of cognitive impairment by relative abundance of bacterial taxa or pathways identified in the ANCOM-II analysis of global cognitive impairment, adjusting for the same covariates as in the ANCOM-II models. Centered log-ratio (CLR) transformation in relative abundances of taxonomic units or functional pathways were used. A pseudocount of min (relative abundance)/2 was added to exact zero relative abundance before taking logs. As a secondary

analysis, we also estimated the OR of impairment in each domain by relative abundance of the identified taxa or pathways associated with the primary outcome (i.e., overall cognitive impairment) in the ANCOM-II analysis. In addition to the primary analysis among all women, we also carried out the analysis stratified by HIV serostatus. To test potential effect modification by HIV serostatus, we included a product term of taxa and HIV serostatus in the regression models. For bacteria found to be associated with cognitive impairment, relative abundance between HIV+ and HIV− women was compared using Wilcoxon rank-sum test. We examined the associations of the identified taxa with global and domain-specific T scores as continuous cognitive outcomes, using linear regression. We also conducted sensitivity analysis among women who had stool sample collection and neuropsychological test at the same visit. We assessed correlations between identified taxa and MetaCyc pathways using CLR transformed relative abundance and spearman correlation coefficient. Analyses were performed using R 4.0.3. A two-sided $P<0.05$ was considered statistically significant in regression models.

Results

Table 1 shows characteristics of the 446 women (302 HIV +, mean age 53.1 years). Cognitive impairment was identified in 122 (27.4%) women. As compared with HIV− women, HIV+ women were slightly older, were more likely to be non-Hispanic white, current smoker and have education below high school, and were less likely to have recreational drug use and marijuana use. We observed a higher percent of global cognitive impairment and impairment in most domains except for memory among HIV+ women as compared with HIV− women. However, most of these differences were not statistically significant.

Associations of gut microbiome alpha diversity and beta diversity with global cognitive impairment

Gut microbiome alpha diversity as measured by Shannon index and number of observed ASVs were higher among women with cognitive impairment as compared to those without ($P\leq0.001$). The associations were consistent among HIV+ women. (Figure S1) No difference in alpha diversity by cognitive impairment status was found among HIV− women. (Figure S1) No difference in beta diversity as measured by Jensen-Shannon Divergence (JSD) was found in HIV+ or HIV− women using PERMANOVA and PCoA (R^2=0.47%-0.59%, $P>0.05$, Figure S2).

Associations of microbial taxa with global cognitive impairment

A total of 7 genera and 5 species showed greater abundance while 1 genus showed lower abundance among women with cognitive impairment as compared to women without, using the ANCOM-II method adjusting for covariates (detection level ≥ 0.60, FDR $q<0.10$). The identified taxa included *Methanobrevibacter*, *Pyramidobacter*, *Gemmiger*, *Ruminococcus*, *Eubacterium*, *Odoribacter*, *Veillonella*, *Pyramidobacter piscolens*, *Ruminococcus bromii*, *Ruminococcus cadillus*, *Gemmiger formicilis* and *Eubacterium biforme*. (Figure 1) The relative abundances of *Methanobrevibacter* and *Eubacterium biforme* were lower while the

relative abundance of *Ruminococcus bromii* was higher among HIV+ women as compared with HIV− women. (P<0.05, Figure S3)

The ORs of cognitive impairment decreased with higher abundance of *Veillonella* while increased with higher abundance of other taxa. (Figure 2) The associations of most taxa, except for *Methanobrevibacter* and *R. cadillus*, with cognitive impairment, were more profound in HIV+ women, and there were significant interactions (P<0.03) for *Eubacterium*, *Veillonella, E. biforme* and *R. bromii*. (Figure 2) The results were generally consistent in analysis using global T score to define cognitive outcomes. (Figure S4) Sensitivity analysis among women with concurrent stool sample collection and cognitive assessment showed similar results. (Table S1)

Associations of microbial taxa with impaired cognitive domains

For individual bacterial taxa, we found associations of most taxa with impairment in learning and memory, with directions consistent with those for global cognitive impairment. (Figure 3) There were also suggestive associations of taxa except for *E.biforme* and *R.callidus* with psychomotor speed and verbal function. (Figure 3) Most taxa were not associated with cognitive impairment in attention, executive function or motor function. (Figure 3) We also examined these associations in HIV+ and HIV− women separately, and most of these findings were predominantly observed in HIV+ women. (Table S2) When using domain T scores, the directions of the associations were largely consistent with the above findings in most domains except for executive function and motor function. (Figure S5) Higher abundance of *Ruminococcus, R. bromii* and *Methanobrevibacter* were associated with lower T scores for learning, memory, psychomotor speed and verbal function. In addition, higher abundance of *Odoribacter, Gemmiger, G.formicillis, Pyramidobactor, P.piscolens* were associated with lower T scores for memory. Unlike null findings with cognitive impairment in executive function and motor function, we found that most taxa were inversely associated with T scores for executive function and motor function. (Figure S5)

Associations of microbial metabolic pathways with global cognitive impairment

We identified 12 functional pathways that were enriched and 3 pathways that were depleted in women with cognitive impairment using ANCOM-II, adjusting for covariates. (Table S3) Ten of them, which were Archaea related pathways or methanogenesis pathways, were highly correlated with *Methanobrevibacter* (Spearman's correlation coefficient>0.80). (Figure 4) *Eubacterium* had a strong correlation with lactose and galactose degradation I (r=0.56). (Figure 4) *Veillonella* was negatively correlated with most pathways, whereas reductive acetyl coenzyme A pathway, super pathway of glycolysis and Entner-Doudoroff, and tricarboxylic acid (TCA) cycle VII pathway were inversely correlated with most taxa. (Figure 4) The latter three pathways were mainly correlated with *Ruminococcus* and *Gemmiger* (r=−0.15 ~ −0.27). (Figure 4) In addition, they were inversely associated with cognitive impairment while other pathways were positively associated with cognitive impairment. (Table S3). The findings of super pathway of TCA cycle VII, and lactose and galactose degradation I pathways were only seen in HIV+ women while the findings of other pathways seemed to be present in both HIV+ and HIV− women. (Table S3)

Discussion

To the best of our knowledge, this is the first large observational study examining the associations between GMB and cognitive function in the context of HIV infection. In this cross-sectional study of middle-aged women with and without HIV who were comparable in demographic and socioeconomic status, we observed that higher microbial alpha diversity and abundances of 7 genera and 5 species were associated with cognitive impairment. Findings of increased risk for cognitive impairment with higher abundance of *Pyramidobacter*, *Eubacterium*, *Ruminococcus*, and *Gemmiger*, and lower abundance of *Veillonella* were mainly observed among HIV+ women, most of whom had undetectable viral load because of long-term ART use. Most of the identified taxa were related specifically to cognitive impairment in learning, memory and processing speed.

Previous studies have linked alterations in GMB with neurodegenerative disorders in animal models, and in humans through gut dysbiosis and inflammatory response. [10, 11, 13, 32-36] However, according to a recent review, findings were not consistent across published studies.[37] Although our findings suggest that GMB was altered among HIV+ women with cognitive impairment, especially in learning and memory domains, they are not fully consistent with published findings on correlates of cognitive impairment in the general population. We identified several genera that have been reported in literature in association with cognitive impairment, including *Eubacterium*, *Odoribacter*, *Ruminococcus*, and *Veillonella*. *Eubacterium*, *Odoribacter* and *Ruminococcus* produce SCFA through fermentation of carbohydrates while *Veillonella* utilizes lactate.[14, 38-41] The role of SCFA in inflammation has not been well elucidated, which may partially explain why there have been mixed findings in literature about the roles of these taxa in inflammation. SCFA are mainly believed to be anti-inflammatory, but may also exhibit multiple effects in leucocyte recruitment and chemokine production under different conditions and in different types of cells. SCFA may be pro-inflammatory when there is bacterial infection or damage of gut epithelium, or in microglial cells.[42] Furthermore, in contrast to the anti-inflammatory role of butyrate, acetate seems to be involved in cytokine production, which might be implicated in amyloid deposition in the brain relating to cognitive dysfunction and Alzheimer's disease. [43]

Consistent with our findings, higher abundance of *Odoribacter* has been shown among patients with Alzheimer's disease and mild cognitive impairment in comparison to people with normal cognitive function. [14, 44] Notably, its genes have been connected with Alzheimer's disease pathway in Kyoto Encyclopedia of Genes and Genomes. [14, 45] In this study, *Odoribacter* was positively associated with impaired memory among HIV+ women, which has not been reported before, to the best of our knowledge. Evidence linking altered abundance of *Eubacterium*, *Ruminococcus* and *Veillonella* to cognitive impairment are mixed. Similar to what we found, some studies reported depleted *Veillonella* and enriched *Eubacterium* species, *E. eligens*, among patients with mild cognitive impairment,[13] while other studies reported opposite results for *Veillonella* with mild cognitive impairment, and *E. eligens*, *E. hallii*, and *E. rectale* among those with Alzheimer's disease. [11, 14, 46] Interestingly, growth of *E. rectale* is stimulated by *R. bromii*, so it is not surprising to see both taxa showed positive association with cognitive impairment. [40, 47] *Ruminococci*

are species with high abundance in the human intestines.[48] Some studies found lower abundance of *Ruminococcus* in patients with Alzheimer's disease and mild cognitive impairment and a positive correlation of this genus with better naming function, and working memory. [11, 46] Yet, it has also been reported to be pro-inflammatory through its role in intestinal immune response.[49, 50] Nevertheless, findings from prior work and the current study support a link of GMB alteration (e.g., increased abundance of *Odoribacter*) with cognitive impairment and Alzheimer's disease.

We also identified three genera that have not been previously related to neurocognitive disorders in prior literature, to the best of our knowledge, namely, *Gemmiger*, *Methanobrevibacter* and *Pyramidobacter*. *G. formicilis* has been observed to be lower in abundance among men with HIV who progressed to acquired immunodeficiency syndrome (AIDS) comparing to those who stayed AIDS-free for 10 years without using antiretroviral therapy.[51] It has also been associated with longer survival and developing colitis when using ipilimumab to treat patients with metastatic melanoma.[52] *P.piscolens* has been enriched in patients with chronic periodontitis, ischemic stroke, and low-set rectal cancer patients after FOLFOX treatment.[53-55] *Pyramidobacter* belongs to phylum Synergistes, which may play a pathogenic role in infections.[56, 57] *Methanobrevibacter* is a dominant archaea commonly found in healthy people, with *M.smithii* being the major species.[58, 59] *M.smithii* facilitates production of acetate, butyrate and ATP by removing dihydrogen from host gut environment.[58] It has been implicated in obesity, severe acute malnutrition in children, colorectal cancer, anorexia, inflammatory bowel disease, irritable bowel disease, diverticulosis, constipation and periodontitis.[58, 60] These three genera appear to share the common link to disease pathology involving immune reactions and inflammatory response. We speculate that they may play a role in the development of cognitive disorders in PLWH, who may be subject to chronic immune activation.[61] Future research are needed to understand the underlying mechanisms.

Among PLWH, HIV virus causes gut mucosa damage and translocation of proinflammatory microbial products such as lipopolysaccharide, which induces the release of cytokines in CNS, resulting in neuroinflammation.[61, 62] In line with this, we found that the associations between gut microbial features and cognitive impairment were more profound among HIV+ women. However, it should be noted that the non-significant results in HIV− women might be due to relatively smaller sample size in this group. In addition, our prior work has found enriched *Ruminococcus* genus in HIV+ women compared to HIV− women.[31] In the current study, we observed enriched abundance of *R. bromii* associated with both HIV infection and cognitive impairment. This supports a hypothesis that HIV infection may lead to altered GMB profile, which subsequently contributes to the development of cognitive impairment. Furthermore, PLWH are more prone to have systemic inflammation and are at higher risk of aging related disease such as cardiovascular disease, and neurological disorders compared to those without HIV.[61-64] Of note, among the genera we found, *Eubacterium*, *Methanobrevibacter,* and *Pyramidobacter*, have also been associated with aging.[65] Aside from a distinct study population of PLWH, another explanation for inconsistent findings between this study and other existing studies could be the different measures of cognitive functions and endpoints used across studies. Some studies used Alzheimer's disease and dementia as endpoints, which are more severe forms

of cognitive impairment. Even among studies investigating mild cognitive impairment, most studies did not assess cognitive function using seven domains. A majority of women in the present study only demonstrated mild cognitive impairment. Such conflicting results by disease severity has been seen in *Bacteroides* and *E. eligens*, which were found to be depleted in patients with Alzheimer's disease but increased among people with mild cognitive impairment. [13, 14, 35, 36]

Several papers examined potential mechanisms through analysis of microbial gene pathways. They have identified increased glycan biosynthesis and metabolism, transport and catabolism, and vitamin B metabolism, and depleted butyrate biosynthesis pathways, transcription and membrane transport.[11, 14, 46] In our analysis, most pathways related to cognitive impairment were connected to *Methanobrevibacter*. For example, Coenzyme B and tetrahydromethanopterin are coenzymes in methanogensis while wyosine, CDP-archaeol and archaetidylinositol biosynthesis pathways are related to archaea, which are methanogens.[66] We found pathways depleted in cognitive impairment related to generation of cell energy and precursors of metabolites, including TCA cycle VII (acetate producers) and superpathway of glycosis and Entner-Doudoroff, and pathways enriched in cognitive impairment related to carbohydrate degradation pathway (lactose and galactose degradation I). In addition, we found that pathways related to biosynthesis, including the mevalonate pathway, an amino acid related pathway (chorismate biosynthesis II) and a vitamin related pathway (flavin biosynthesis II), were enriched among women with cognitive impairment. These pathways play fundamental roles in biosynthesis of isoprenoids (mevalonate pathway), aromatic amino acids such as phenylalanine, tryptophan and tyrosine, indole, vitamin K, and folate (chorismate pathway).[66] Riboflavin is an essential nutrient that mammals cannot synthesize, and has been linked with potential antioxidant and neuroprotective effects.[67, 68] Similar to our findings, a recent study also reported worse functioning in memory domains in association with increased bacterial vitamin metabolism related pathways including riboflavin, vitamin B6, folic acid, vitamin B1, and vitamin B12.[46] The authors hypothesized that as a result of bacteria competing for vitamins, host uptake of vitamins were limited. [46] In contrast, it is possible that bacterial vitamin pathways are enriched in cognitive impairment because the host may be vitamin deficient, resulting in cognitive impairment and necessitating bacterial synthesis of vitamins. More studies are needed to replicate our findings and explore these hypotheses.

Strengths and Limitations

To the best of our knowledge, this is the first study with a considerable sample size to examine GMB alterations in association with cognitive impairment among women living with HIV, with a comparison group of uninfected women. We conducted comprehensive assessment of cognitive functions of seven domains and explored microbiome composition change in relation to these functions in each domain. We included a comparison group of women at risk of HIV, who were comparable in demographics, socio-economic status and health-related behaviors as women with HIV. Such a design allowed us to evaluate and interpret the findings more meaningfully. We examined both microbiota compositional changes and metagenomic prediction of functional changes, which may shed light on the roles of GMB in cognitive disorders among PLWH.

Limitations included a cross-sectional study design, and thus we were unable to assess temporal relationships and establish causation between GMB and cognitive impairment. Future studies are needed to examine GMB in association with changes in cognitive outcomes. Second, stool sample collection was not always concurrent with cognitive function measurement. However, most (80%) of the stool sample collections were completed within 6 months of cognitive data collection, which is nondifferential by HIV status. Our sensitivity analysis has also shown consistent findings among those with GMB data collected at the same visit as cognitive assessment. Third, microbial functional pathways examined in our analysis were inferred based on 16S rRNA gene taxonomic data, and thus require confirmation in the future using shotgun metagenomic sequencing. Fourth, the study may lack power to test effect modification by HIV status due to a relatively smaller sample size of the HIV− group. Cognitive impairment was also defined based on data from this small group of HIV− women in the current analysis. Moreover, we did not collect diet and lifestyle data and cannot account for influences of these factors on GMB and cognitive function. Finally, generalization of the results from this study may be restricted to women living with HIV in the urban areas of northeast US, who were engaged in HIV research and under long-term HIV care.

Conclusions

This study provides evidence for the associations of the GMB with cognitive impairment among women with HIV. More studies are needed to validate these findings, understand the underlying mechanisms, and assess the potential of the GMB to become biomarkers and/or therapeutic targets in cognitive diseases including Alzheimer's disease among PLWH. Further examinations of metabolites such as SCFA, and enzymes in bacterial functional pathways may deepen the understanding of the complicated biological mechanisms linking the GMB with cognitive function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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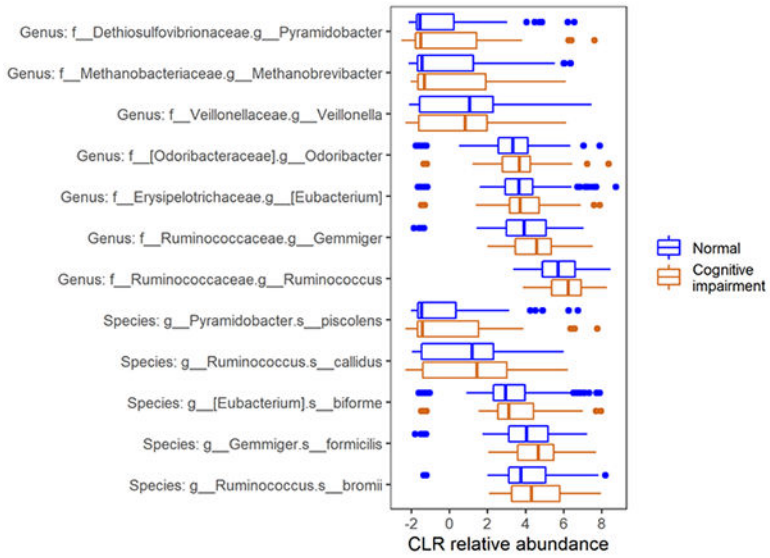

Figure 1. Relative abundance of taxa identified using ANCOM2 by cognitive impairment

Taxa were identified using ANCOM-II adjusting for age, race, education, poverty, recreational drug use, HCV, site, antibiotics, smoking, viral load and ART use (among HIV+) as appropriate. Central-log-ratio-transformed relative abundance are shown among women with cognitive impairment and women with normal cognitive function. Taxa were ordered by relative abundance at genus and species level, respectively.

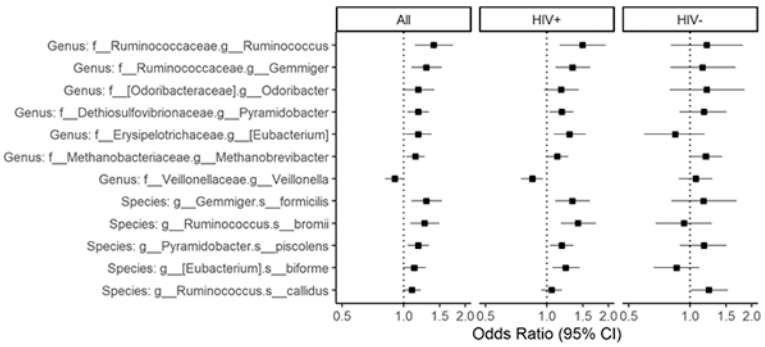

Figure 2. Association between taxa abundance and cognitive impairment among all women and according to HIV serostatus

Taxa were identified using ANCOM-II adjusting for age, race, education, poverty, recreational drug use, HCV, site, antibiotics, smoking, viral load and ART use (among HIV+) as appropriate. Logistic regression was used to show OR of cognitive impairment by relative abundance of taxa, adjusting for above covariates used in ANCOM-II. Relative abundance were CLR-transformed. P for interaction<0.05 for *Eubacterium, Veillonella, Eubacterium biforme and Ruminococcus bromii*. Taxa were ordered by OR at genus and species level, respectively.

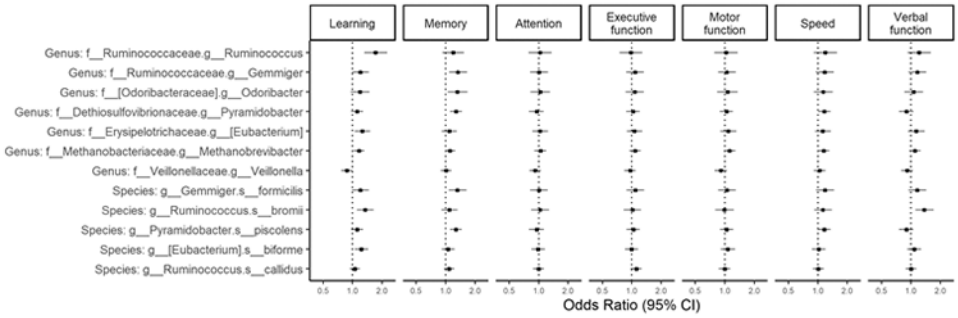

Figure 3. Association between taxa abundance and impairment in cognitive domains among all women

Taxa were identified using ANCOM-II adjusting for age, race, education, poverty, recreational drug use, HIV, HCV, site, antibiotics, smoking, viral load and art use (among HIV+) as appropriate. Logistic regression was used to show OR of cognitive impairment by relative abundance of taxa, adjusting for above covariates used in ANCOM-II. Relative abundance were CLR-transformed.

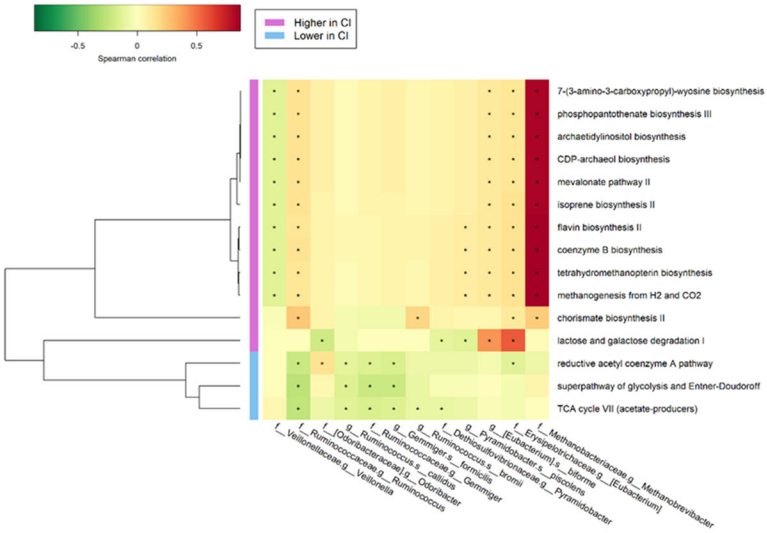

Figure 4. Spearman's correlation coefficients between taxa and pathways identified using ANCOM-II

*: P<0.05 for spearman correlation coefficient.

Table 1.

Characteristics of WIHS participants by HIV status

	All (N=446)	HIV+ (N=302)	HIV− (N=144)	P-value
Age, years, mean (SD)	53.1 (8.3)	53.2 (7.8)	52.8 (9.3)	0.59
Race/ethnicity, %				0.30
Non-Hispanic black	323 (72.4)	214 (70.9)	109 (75.7)	
Hispanic	92 (20.6)	62 (20.5)	30 (20.8)	
Non-Hispanic white	19 (4.3)	16 (5.3)	3 (2.1)	
Other	12 (2.7)	10 (3.3)	2 (1.4)	
Education attainment, %				0.84
Below high school	180 (40.4)	124 (41.1)	56 (39.2)	
High school	141 (31.7)	93 (30.8)	48 (33.6)	
Above high school	124 (27.9)	85 (28.1)	39 (27.3)	
Years of education	11.6 (3.0)	11.7 (3.1)	11.5 (3.0)	0.56
WRAT-3 reading subtest, mean (SD)	87.8 (18.6)	88.0 (19.1)	87.5 (17.8)	0.81
Annual income ≤$12000, %	235 (52.7)	154 (51.0)	81 (56.2)	0.35
Recreational drug use, %	105 (23.5)	66 (21.9)	39 (27.1)	0.27
Marijuana use, %	90 (20.2)	56 (18.5)	34 (23.6)	0.26
Cigarette use, %				0.10
Current smoker	181 (40.6)	74 (24.5)	24 (16.7)	
Former smoker	167 (37.4)	114 (37.7)	67 (46.5)	
Never smoker	98 (22.0)	114 (37.7)	53 (36.8)	
Alcohol use>7 drinks/week, %	30 (6.7)	18 (6.0)	12 (8.3)	0.46
Antibiotic use, %	23 (5.2)	15 (5.0)	8 (5.6)	0.97
Hepatitis C virus antibody, %	97 (21.8)	70 (23.3)	27 (18.8)	0.34
Among HIV seropositive				
CD4 count		670 (492-923)		
HIV-1 Viral load≤20 copies/ml, %		224 (74.2)		
ART use, %		277 (91.7)		
Site				0.45
Bronx	185 (41.5)	121 (40.1)	64 (44.4)	
Brooklyn	132 (29.6)	95 (31.5)	37 (25.7)	
Chicago	129 (28.9)	86 (28.5)	43 (29.9)	
Cognitive impairment	122 (27.4)	89 (29.5)	33 (22.9)	0.18
Impairment in domains				
Learning	74 (16.6)	55 (18.2)	19 (13.2)	0.23
Memory	73 (16.4)	47 (15.6)	26 (18.1)	0.60
Attention	62 (14.6)	45 (15.6)	17 (12.5)	0.48
Executive function	67 (15.1)	54 (17.9)	13 (9.2)	0.02
Motor function	62 (14.1)	44 (14.9)	18 (12.5)	0.60
Speed	62 (13.9)	45 (14.9)	17 (11.8)	0.46
Verbal function	60 (13.5)	46 (15.2)	14 (9.7)	0.15

Data are N (%) or mean (SD). SD: standard deviation. WRAT: Wide Range Abilities Test. HIV: human immunodeficiency virus. CD4: cluster of differentiation 4. ART: antiretroviral therapy. Recreational drug use included marijuana, crack, cocaine, heroin and injection drug use. P-values comparing characteristics by HIV status were obtained from t-test for continuous variables, Chi-squared test for categorical