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Investigating the Blood Microbiome in Parkinson's Disease, Schizophrenia, and Posttraumatic Stress Disorder

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Received: 29 October 2024 | **Revised:** 12 May 2025 | **Accepted:** 23 May 2025

Funding: C.R. is supported by the Stellenbosch University Postgraduate Scholarship Programme. C.R., S.S. and S.M.J.H. are supported by the South African Medical Research Council (SAMRC)/Stellenbosch University Genomics of Brain Disorders (GBD) Extramural Unit. Research reported in this study was supported by the South African Medical Research Council for the "Shared Roots" Flagship Project, Grant MRC-RFA-IFSP-01-2013/SHARED ROOTS through funding received from the South African National Treasury under its Economic Competitiveness and Support Package. The work by L.L.v.d.H was supported in part by the National Research Foundation of South Africa (Grant 138430) and by the South African Medical Research Council under a Self-Initiated Research Grant.

Keywords: Kraken2 | neuropsychiatric disorders | RNA-sequencing

ABSTRACT

Introduction: Recent studies have challenged the idea of sterile blood, suggesting the presence of a blood microbiome. The detection of microbial nucleic acids in blood is thought to reflect the status of distant microbial niches, including the gut. However, the origins and viability of these microbes remain debated. In this study, blood microbiome signatures in Parkinson's disease (PD), schizophrenia (SCZ), and posttraumatic stress disorder (PTSD) were evaluated by extracting RNA-sequencing (RNA-seq) reads that did not map to the human genome. Furthermore, we investigated the correlation between the gut and blood microbiome in PD, SCZ, and PTSD to gain insight into possible mechanisms behind disease etiology.

Methods: We used whole-blood samples from PD (cases: $n = 14$; controls: $n = 19$), SCZ (cases: $n = 17$; controls: $n = 22$), and PTSD (cases: $n = 45$; trauma-exposed [TE] controls: $n = 34$) cohorts. The RNA paired-end sequence reads that did not map to the human reference genome (hg38/GRCH38) were isolated using the sequence alignment/map tools (SAMtools). These unmapped reads were classified against known archaeal, bacterial, and viral microbial genomes using *Kraken2* (v2.1.3; k2_standard_08gb_20240112.taz.gz database), and further taxa abundances were estimated using *Bracken* (v2.9). The differential abundance of blood microbial signatures between case-control groups for each cohort was assessed using *DESeq2* (v1.38.3). Each cohort was analyzed separately.

Results: Statistically significant differences in the abundance of *Pseudomonas aeruginosa* and *Acinetobacter wuhouensis* in PD and *Salmonella enterica*, *Staphylococcus aureus*, *Pseudomonas* sp. CC6-YY-74, and *Shinella sumterensis* in SCZ were observed compared to cohort-specific controls. We observed no statistically significant differences in microbial signatures between PTSD cases and controls.

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Conclusion: We found blood microbial signatures associated with PD and SCZ; however, no significant blood microbial signature was observed for PTSD. These results should be interpreted with caution as biases may have been introduced due to low concentrations of microbial signatures. Further research is required to understand the biological implications of these findings, in particular, taking into account the repurposed data source, and the potential for contamination (during phlebotomy, and background contamination from DNA extraction and reagents) known to impact the analysis of low-biomass samples.

1 | Introduction

The idea of blood as sterile has been challenged in recent years (Cheng et al. 2023; Hodzhev et al. 2023; Jagare et al. 2023; Sciarra et al. 2023), as approximately 35.2% (2.5% of total reads) of whole genome and transcriptome data not mapped to the human genome can be assigned to microorganisms (Amar et al. 2013; Damgaard et al. 2015; Potgieter et al. 2015; Loohuis et al. 2018; Whittle et al. 2019; Cheng et al. 2023). The detection of microorganisms and their genetic material in both healthy and disease groups (Amar et al. 2013; Potgieter et al. 2015; Païssé et al. 2016; Loohuis et al. 2018; Adams et al. 2019; Serena et al. 2019; Whittle et al. 2019) has contributed to the conceptualization of the blood microbiome (Cheng et al. 2023). However, this detection of microorganisms in the blood is unable to determine whether the genetic material originated from viable or dead microbiota or microbial cell-free nucleic acid (Damgaard et al. 2015; Païssé et al. 2016; Castillo et al. 2019; Suparan et al. 2022). As this contributes to more uncertainty and controversy, the term "blood microbiome" is preferred over blood microbiota, as the microbiome refers to the collective genome of microorganisms (Suparan et al. 2022). Controversy regarding the blood microbiome is further supported by the idea that the immune system typically eliminates viable microorganisms from the blood. It has been suggested that blood microbiota remain dormant to evade detection by the immune system and this suggestion is supported by limited bacterial proliferation in healthy blood due to antimicrobial components and scarce free iron (reviewed in Kell and Pretorius 2015; Potgieter et al. 2015).

The origins of the blood microbiome are largely unknown; however, current hypotheses for the origin of the blood microbiome include translocation from other microorganism-rich body sites such as the gut (Jagare et al. 2023) and contamination from the skin or other environmental sources (Figure 1) (Sciarra et al. 2023). Moreover, it is unclear whether the blood milieu represents a permanent niche for the human microbiome or simply functions as a medium for translocation between the other well-established niches of the human microbiome (Jagare et al. 2023; Tan et al. 2023). One hypothesis suggests translocation largely from the gut microbiome to the blood, with the oral microbiome as the second most likely source (Martínez et al. 2022; Suparan et al. 2022; Jagare et al. 2023). Furthermore, it is still debated whether microbes themselves or only their microbial products are translocated to allow the detection of a blood microbiome (Loohuis et al. 2018).

The translocation of microbial products and metabolites from the gut environment into the blood is regulated by the intestinal barrier among others (Chelakkot et al. 2018; Paton et al. 2023), and once in the blood, the translocation of harmful/unwanted substances into the brain environment is regulated by the blood-brain barrier (BBB) (Segarra et al. 2021; Paton et al. 2023). Many soluble microbial metabolites from the gut can enter the bloodstream (Kell and Pretorius 2015, 2018), either through dendritic cells (Goraya et al. 2022) or by crossing the intestinal barrier (Suparan et al. 2022; Jagare et al. 2023). Increased intestinal permeability, often associated with gut microbiome alterations and diseases such as irritable bowel syndrome (IBS) (Suparan et al. 2022; Jagare et al. 2023), can lead to increased translocation of pro-inflammatory metabolites into the blood and ultimately the brain, contributing to inflammation (Paton et al. 2023). The transportation of metabolites between the gut and the brain by the blood plays a crucial role in the gut–brain axis (GBA) (Gwak and Chang 2021) as it supports various GBA functions, including neural, hormonal, and immune responses, and helps regulate the intestinal barrier and the BBB (Valles-Colomer et al. 2019; Bhatia et al. 2023).

High-quality reads obtained from whole blood through RNA sequencing (RNA-seq) contain not only host, but also nonhost, transcriptome data (Wu et al. 2022) that can be used to identify microbial signatures for the blood microbiome in a culture-independent approach (Loohuis et al. 2018). Furthermore, the identified candidates for the blood microbiome can be correlated to other well-established niches of the human microbiome to determine the most likely origin. To date, no core healthy blood microbiome has been established (Cheng et al. 2023; Tan et al. 2023). However, dominant phyla observed in a healthy blood microbiome include Firmicutes, Actinobacteria, Proteobacteria, and Bacteriodetes (Whittle et al. 2019; Goraya et al. 2022; Suparan et al. 2022; Jagare et al. 2023; Sciarra et al. 2023). As discussed, the origin of the translocation of microbiota into the blood is still debated, although several studies reported associations between microbial alterations in the gut microbiome and disease development (Kelly et al. 2015; Chiang and Lin 2019; Loupy and Lowry 2020; Gallop et al. 2021; Andrioaie et al. 2022; Borkent et al. 2022; He et al. 2024). Moreover, several studies on the blood microbiome and neuropsychiatric disorders, such as Parkinson's disorder (PD), schizophrenia (SCZ), and depression have found suggestive associations between microbial alterations and disease development (Loohuis et al. 2018; Qian et al. 2018; Ciocan et al. 2021).

Loohuis et al. (2018) found that the blood microbiome of SCZ cases reflected their gut microbiome and showed increased microbial diversity compared to controls, amyotrophic lateral sclerosis (ALS), and bipolar disorder (BPD) patients. The increased alpha-diversity relating to the blood microbiome of SCZ patients accounted for 5% of disease variation, and higher beta-diversity suggested a general increase in microbial diversity rather than a specific profile (Loohuis et al. 2018). Additionally,

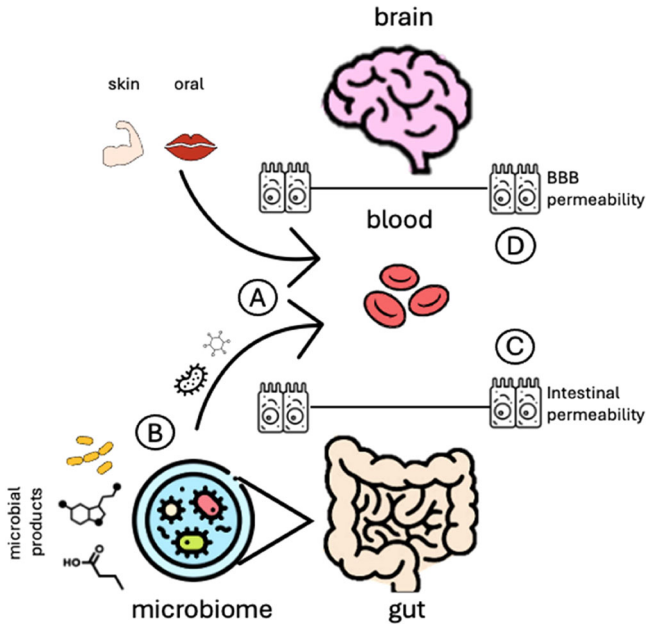

FIGURE 1 | Gut–blood–brain interaction. (A) The origins of the blood microbiome are still unknown. It is suggested that the blood microbiome could be due to the translocation from the gut microbiome and skin or oral microbiome to the blood. (B) Furthermore, it is still debated whether microbes themselves or only their microbial products are translocated to allow the detection of a blood microbiome. (C) The intestinal barrier regulates the translocation of metabolites from the gut environment into the blood. The increase in intestinal permeability could allow the translocation of microbiota and/or their microbial products into the circulation system. The increased levels of microbial products in the blood could contribute to inflammation. (D) The blood-brain barrier (BBB) protects the brain from harmful/unwanted substances in the blood. The dysregulation of the blood microbiome and/or microbial products entering the circulation system could affect the permeability of the blood-brain barrier, allowing inflammatory mediators access to the brain.

two blood phyla, Planctomycetes and Thermotogae, were more prevalent in SCZ patients, but it remains unclear whether these microbial alterations contribute to SCZ or result from the disease (Loohuis et al. 2018).

In PD, the blood microbiome of patients and controls was assessed using 16S rRNA gene sequencing, finding no significant differences in overall diversity or composition between the groups (Qian et al. 2018). However, specific microbiota alterations were associated with clinical characteristics like disease duration and non-motor symptoms, and a random forest model identified 15 genera that could predict PD status, with improved accuracy when combined with gut microbiome data (Qian et al. 2018). However, the study did not support the hypothesis of gut microbiota translocation to the blood in PD, suggesting instead that the blood microbiome might originate from the oral or nasal microbiomes, an association yet to be investigated (Qian et al. 2018). The association between the gut microbiome and the blood microbiome has to the best of our knowledge not been investigated using RNA-seq data derived from human blood in PD (using RNA-seq) or posttraumatic stress disorder (PTSD).

Although the blood microbiome is suggested to reflect the gut microbiome in the SCZ study by Loohuis et al. (2018), it might not be true for other disorder cohorts, as seen in the study on the blood microbiome and PD (Qian et al. 2018). This suggests translocation from other tissue microbiomes into the blood or the role of the intestinal barrier and immune cells in filtering and affecting bacterial translocation from the gut microbiome (Qiu et al. 2019). Moreover, it has been suggested that the blood microbiome might not be conserved in different circulatory compartments or different locations in circulatory systems (Qian et al. 2018). Thus, it would be ideal to combine observations and data from the different microbial communities (e.g., blood, gut, oral, and nasal) as no system is in isolation and alteration in the different systems might present a clearer picture of the etiology of neuropsychiatric disorders and the role of the human microbiome.

The detection of potentially unique disease microbial profiles in the blood does raise the question of underlying mechanisms and how the blood microbiome could contribute to or reflect disease status. Furthermore, whether the potential blood microbial profiles observed in these diseases reflect the gut microbiome. The gut microbiome is now known to have active involvement in the bidirectional communication between the gut, circulation system, and the brain and is associated with brain development, physiology and behavior, and psychology (Valles-Colomer et al. 2019). Therefore, it is important to investigate the microbiome–gut–brain (MGB) interactions beyond 16S rRNA studies and assess taxonomic associations between the gut and blood microbiome through metagenomic data. This could contribute to further insight into underlying mechanisms involving the human microbiome and disease. This current study investigated the association between the gut and blood microbiome in PD, SCZ, and PTSD to elucidate possible overlaps between the blood and gut microbiomes.

2 | Materials and Methods

2.1 | Data and Samples Available

Participants were recruited as part of the parent study, "Understanding the SHARED ROOTS of neuropsychiatric disorders and modifiable risk factors for cardiovascular disease" or "SHARED ROOTS (SR; HREC no. N13/08/115; Department of Psychiatry, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa)". The overall aim of the parent study is to examine signatures (i.e., genomic, neural, cellular, and environmental) commonly associated with neuropsychiatric disorders and cardiovascular disease risk that could contribute to comorbidity, symptom severity, and treatment outcomes. RNA-seq data for the blood microbiome were generated for PD (cases: $n = 14$; controls: $n = 19$), SCZ (cases: $n = 17$; controls: $n = 22$), and PTSD (cases: $n = 45$; trauma-exposed [TE] controls: $n = 34$). The overlap with 16S rRNA (V4) gene sequencing data for the gut microbiome (PD cases: $n = 16$, controls: $n = 42$; SCZ cases: $n = 41$, controls: $n = 48$; PTSD cases: $n = 79$, TE controls: $n = 59$) previously analyzed (Malan-Muller et al. 2022; Rust, van den Heuvel, et al. 2025; Rust, Asmal, et al.2025) is shown in Table 1.

TABLE 1 | RNA-seq data available for each cohort and overlap with 16S rRNA (V4) gene data.

Neuropsychiatric disease	Total	Cases	Controls
Parkinson's disease (PD)			
16S rRNA V4	58	16	42
RNA-seq	33	14	19
Overlap 16S data/RNA-seq data	14	3	11
Schizophrenia(SCZ)			
16S rRNA V4	89	41	48
RNA-seq	39	17	22
Overlap 16S data/RNA-seq data	29	10	19
Posttraumatic stress disorder (PTSD)			
16S rRNA V4	138	79	59
RNA-seq	79	45	34
Overlap 16S data/RNA-seq data	35	26	9

Note: Gut microbiome: 16S rRNA V4; Blood microbiome: RNA-seq.

2.2 | Sample Collection and Preparation

For RNA extraction, 2.5 mL of blood was drawn by means of venipuncture after the skin was cleansed with alcohol for each of the participants. The blood of participants from the PD and PTSD cohorts was drawn by two individuals, both involved in cases and controls, on different days. The blood of participants of the SCZ cohort was all drawn by a single individual on different days. Whole blood was collected in PAXgene tubes (Qiagen, Venlo, Netherlands) from PD (cases: 14; controls: 19), SCZ (cases: 17; controls: 22), and PTSD (cases: 45; controls: 34) participants. The PAXgene tubes were kept at room temperature for no longer than 24 h (minimum of 2 h) before being placed in a -20°C freezer and then eventually stored at 80°C until RNA extraction was performed. The total RNA from the whole blood was extracted using the PAXgene Blood RNA kit (Qiagen, Venlo, Netherlands) in accordance with the manufacturer's protocol. The integrity of the RNA was measured using the Agilent 2100 Bioanalyzer (Agilent Technologies, California, USA).

RNA-seq for all three cohorts was performed at the Kinghorn Centre for Clinical Genomics (KCCG), housed at the Garvan Institute of Medical Research (Sydney, Australia). In accordance with the manufacturer's instructions, the total RNA samples underwent library preparation using Illumina's TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold High (Illumina, California, USA). Sequencing of samples was performed on the Illumina HiSeq 2500 sequencing system (Illumina, California, USA) in a high output mode with V4 chemistry, read length of 125 base pairs (bp), and sequencing depth set at 50 million paired-end reads per sample.

2.3 | Analyses of Microbial RNA Sequences From RNA-seq Data

We repurposed the RNA sequences that were investigated in PD, SCZ, and PTSD by Hemmings et al. (2022) to explore the presence of the blood microbiome in the respective cohorts (PD, SCZ, and PTSD). The hypothesis proposed by Whittle et al. (2019) is that reads from the RNA-seq of human blood plasma failing to map to the host genome (hg38/GRCH38 human genome) may convey the presence of microbial nucleic acid present in human plasma at the time of RNA extraction.

2.4 | Data Preparation

The high-quality raw sequencing data (length of 125 bp, sequenced at a depth of 50 million paired-end reads per sample) for all three cohorts underwent FASTQ quality control (QC), and adaptors were trimmed using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit). Multipart FASTQ files of single reads were concatenated into single file (compressed version of a FASTQ dataset: fastq.gz files). The RNA paired-end sequence reads were then aligned to a human reference genome (hg38/GRCH38) using hierarchical indexing for spliced alignment of transcripts (HiSAT2) version 2.2.1 (Kim et al. 2019). The sequence alignment/map tools (SAMtools) were then used to isolate unmapped reads that did not map to the human reference genome, resulting in binary alignment/map (BAM) files, whereafter pair-end reads were isolated with the *bamToFastq* function in BEDtools version 2.31.1.

Only sex and age were included as variables of interest due to the exploratory nature of the study and to not overcomplicate the model. Moreover, the addition of each covariate to the model reduces the degrees of freedom, resulting in a diluted statistical power. Differences between case and control of the respective PD, SCZ, and PTSD cohort for sex were assessed using the chi-square test. Normality was determined by the Shapiro–Wilk test (normality: p-value > 0.05). Age was normally distributed and the t-test was used to determine differences between cases and controls, with the results expressed in the form of the mean and standard deviation (SD). All the test results were considered statistically significant at the threshold of less than 0.05.

2.5 | Taxa Classification and Taxa Abundance Estimations

All unmapped reads were matched against known archaeal, bacterial, and viral microbial genomes using a Kraken algorithm, a very fast read-level taxonomic classifier (Poore et al. 2020). The number of unmapped reads per sample that mapped to the four major domains (Eukaryota, Bacteria, Archaea, and Viruses) for each cohort is shown in Table S1. The archaeal and bacterial genomes are usually filtered with a QC of 0.8 or higher (Poore et al. 2020). The sample sequence reads were broken up into k-mers by Kraken, in order to match them to the microbial database of k-mers built from microbial genomes to identify the taxonomy of the unmapped reads (Poore et al. 2020). This provides a putative taxonomy identification of the assembly at the lowest common ancestor (most accurate at the

genus level). For classification, Kraken2 (version 2.1.3) was used to classify samples against the k2_standard_reference database (k2_standard_08gb_20240112.tar.gz; https://benlangmead.github.io/aws-indexes/k2) to generate Kraken reports (kreports) (Wood and Salzberg 2014; Wood et al. 2019) as Kraken2 specifies five ranks (superkingdom, order, phylum, family, genus, and species). The resulting "kreports" from samples were combined into a single report using the publicly available *combine_kreports.py* script available from KrakenTools on GitHub (https://github.com/jenniferlu717/KrakenTools#combine_kreportspy).

Subsequent to Kraken2 classification, the Bayesian Reestimation of Abundance after Classification with Kraken (Bracken; version 2.9) was used to estimate abundance at the species and genus level using the taxonomic assignments made by Kraken2 with the same k2_standard_reference (https://benlangmead.github.io/aws-indexes/k2) database (Lu et al. 2017; Buffet-Bataillon et al. 2022). The bracken report files for each sample were combined into one report based on genus or species classification using the publicly available *combine_bracken_outputs.py* script (https://github.com/jenniferlu717/Bracken/blob/master/analysis_scripts/combine_bracken_outputs.py).

2.6 | Differential Abundance of Blood Microbial Taxa

Differential abundance was assessed with DESeq2 (available from Bioconductor online at https://www.bioconductor.org/packages/release/bioc/vignettes/DESeq2/inst/doc/DESeq2.html and https://bioconductor.org/packages/devel/bioc/manuals/DESeq2/man/DESeq2.pdf; version 1.38.3) After importing the count table obtained from the Bracken analysis, data were filtered to have at least one read per taxa and the library size was estimated as scaling factor to normalize data (*estimateSizeFactors* function) for DESeq2 (Love et al. 2014). The count data were converted to $\log_2$ scale to allow visualization (Table S2). Additional quality assessments based on sampling clustering were done using a heatmap (*pheatmap* function). Differences in sample distances for cases compared to controls were assessed with the principal coordinate analysis (PCA) plot (https://bioconductor.org/packages/release/workflows/vignettes/rnaseqGene/inst/doc/rnaseqGene.html#pca-plot). Differential expression analysis was completed (*DESeq* function) with statistical significance set as 0.05 for the adjusted *p*-value to correct for multiple testing. Statistically significant taxa were visualized using *pheatmap*, including sex and age. Additional statistically significant taxa were plotted using *ggplot* function to show differences between means for cases versus controls.

2.7 | Correlation Between Gut and Blood Microbiome

The participants with both the 16S rRNA (V4) gene (stool sample) and RNA-seq (blood sample) data were used to investigate correlations between the gut and blood microbiome. From the available data, 77 participants had microbial data for both the gut and blood microbiome, including 14 PD participants (case: 3, control: 11), 29 SCZ participants (case: 10, control: 19), and 35 PTSD participants (case: 26, control: 9) shown in Table 1.

The correlation between the gut and blood microbial profiles was assessed with the Procrustes analysis (Jackson 1995) by comparing results from the principal component analysis (PCA) using the *vegan* package (version 2.6-4) in R studio (version 2023.06.1+524). Procrustes analysis rotates the first matrix (e.g., PCA for blood microbiome abundance data) to maximum similarity with the second matrix (e.g., PCA for gut microbiome abundance data) by minimizing the sum of squared differences (Jackson 1995).

The *janitor* package (version 2.2.0) in R studio (version 2023.06.1+524) was used to identify specific taxa present in both the gut and blood microbial data. The identified taxa were assessed for correlations between the abundances in the gut and the blood. Spearman's correlation test was used to measure the strength and direction of the correlation of the taxa abundance between the gut and the blood microbiome. The correlation was considered statistically significant at a threshold of less than 0.05.

3 | Results

3.1 | Cohort Description

Descriptions of the PD, SCZ, and PTSD cohorts are shown in Table 2. We observed no statistically significant differences for sex (PD $p = 0.254$; SCZ $p = 0.922$; and PTSD $p = 0.374$) or age (PD $p = 0.604$; SCZ $p = 0.625$; and PTSD $p = 0.337$) between cases and controls within the respective cohorts (Table 2).

3.2 | PD and the Blood Microbiome

Through the use of the DESeq2 analysis, we found PD to be associated with the enriched signature of *Mycobacterium* sp. Z3061 ($p = 0.009$; $q = 0.179$), *Clostridium baratii* ($p = 0.022$; $q = 0.300$), *Vibrio mediterranei* ($p = 0.030$; $q = 0.300$), *Acinetobacter wuhouensis* ($p < 0.000$; $q < 0.000$), *Laribacter hongkongensis* ($p = 0.037$; $q = 0.325$), and *Serratia grimesii* ($p = 0.029$; $q = 0.300$), and the depleted signature of *Pseudomonas aeruginosa* ($p < 0.000$; $q < 0.000$)(Table 3). However, only *P. aeruginosa* and *A. wuhouensis* withstood correction for multiple testing. Of the total unmapped reads for the PD cohort (389,961,750), *P. aeruginosa* and *A. wuhouensis* each represented 80 (< 0.0% of total unmapped reads) and 16 (< 0.0% of total unmapped reads) total reads respectively. Furthermore, all control participants and a single PD case (7.1%) had blood microbial signatures for *P. aeruginosa*. All control participants and three PD cases (21.4%) had blood microbial signatures for *A. wuhouensis*. No clear clustering of differential taxa expression due to age or sex was observed (Figure 2).

3.3 | SCZ and the Blood Microbiome

We observed a statistically significant enriched signature for *Salmonella enterica* ($p < 0.000$; $q < 0.000$) and a statistically significant depleted signature for *Staphylococcus aureus* ($p < 0.000$; $q < 0.000$), *Pseudomonas* sp. CC6-YY-74 ($p < 0.000$; $q <$

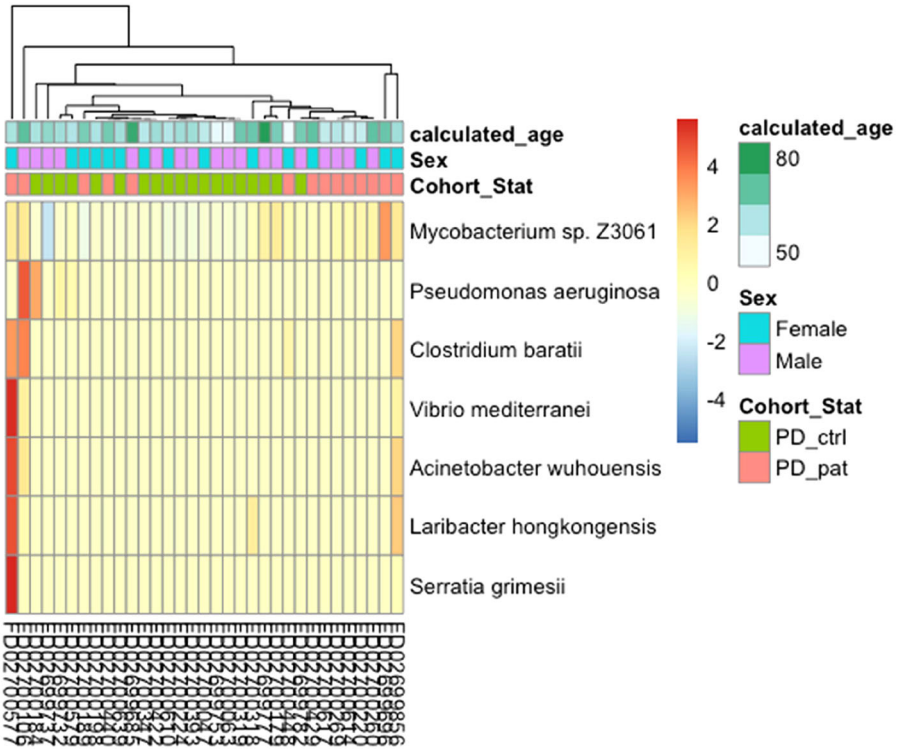

FIGURE 2 | Heatmap for statistically significant enriched signature in PD cases compared to controls. The heatmap shows the statistically significant taxa that were observed to be different between PD cases compared to controls. Only *Pseudomonas aeruginosa* ($p < 0.000$; $q < 0.000$) and *Acinetobacter wuhouensis* ($p < 0.000$; $q < 0.000$) withstood correction for multiple testing. The heatmap indicates in which samples the taxa abundance was enriched or depleted. Moreover, it indicates whether samples were female or male, and their age. No clear clustering of differential taxa expression was observed due to age or sex.

0.000), and *Shinella sumterensis* ($p = 0.002$; $q = 0.033$) in SCZ cases compared to controls (Table 3). All the abovementioned observations survived correction for multiple testing. Of the total number of unmapped reads for the SCZ cohort (450,836648), *S. enterica* was represented by 734 total reads (0.0002% of total unmapped reads), *S. aureus* by 379 total reads (0.0001% of total unmapped reads), *Pseudomonas* sp. CC6-YY-74 by 318 total reads (0.0001% of total unmapped reads), and *S. sumterensis* by 22 total reads (< 0.0% of total unmapped reads). Furthermore, all participants had blood microbial signatures for *S. aureus*, whereas only seven participants (17.9%) had blood microbial signatures for *S. sumterensis* and *Pseudomonas* sp. CC6-YY-74. *S. enterica* was only present in the blood microbiome of four participants (10.5%). Based on the heatmap in Figure 3, there was no clear clustering based on age.

3.4 | PTSD and Blood Microbiome

In the PTSD cohort, we observed no statistically significant microbial signatures in the blood of cases compared to controls before ($p < 0.05$) or after correction for multiple testing ($q < 0.05$).

3.5 | Correlation Between Gut and Blood Microbiome

The results from the Procrustes analysis suggested that the two microbiome environments significantly differed for all three cohorts (PD $p = 0.995$; SCZ $p = 0.291$; PTSD $p = 0.580$). The correlation between the taxa abundance of these taxa in the blood and gut microbiome is shown in Table 4, as well as the percentage of cases and controls with microbial signatures for the specific taxa. *Clostridium* and *Streptococcus* were present in the gut and blood microbiome of all three cohorts. *Lactobacillus* was present in the gut microbiome of the PD cohort and only in the blood microbiome of PD cases. *Bacteroides* was observed in the gut and blood microbiome of the PD and SCZ cohorts. *Prevotella* was only present in both microbiomes of the PTSD cohort. *Enterococcus* and *Fusobacterium* were only present in the gut and blood microbiome of the SCZ cohort. The abundance of *Campylobacter* present in the gut and blood microbiome of the SCZ cohort was the only taxa to significantly correlate between the two environments ($p = 0.036$). *Bacteroides, Fusobacterium*, and *Campylobacter* were only present in SCZ cases and not in controls. The correlation between the abundance of the taxa in the gut and blood could not be calculated if the SD of one of the environments was 0, as indicated, as it would result in no second variable to calculate a correlation (Moore et al. 2016).

We further evaluated if any of the correlating taxa were previously associated with case-control status in the gut microbiome (Malan-Muller et al. 2022; Rust, van den Heuvel, et al. 2025; Rust, Asmal, et al. 2025). The decreased relative abundance of *Clostridium* in the gut was previously associated with PD in our cohort (Rust, van den Heuvel, et al. 2025). The decreased relative abundance of *Prevotella*, on the other hand, was associated with SCZ case-control status in our cohort, although it did not withstand

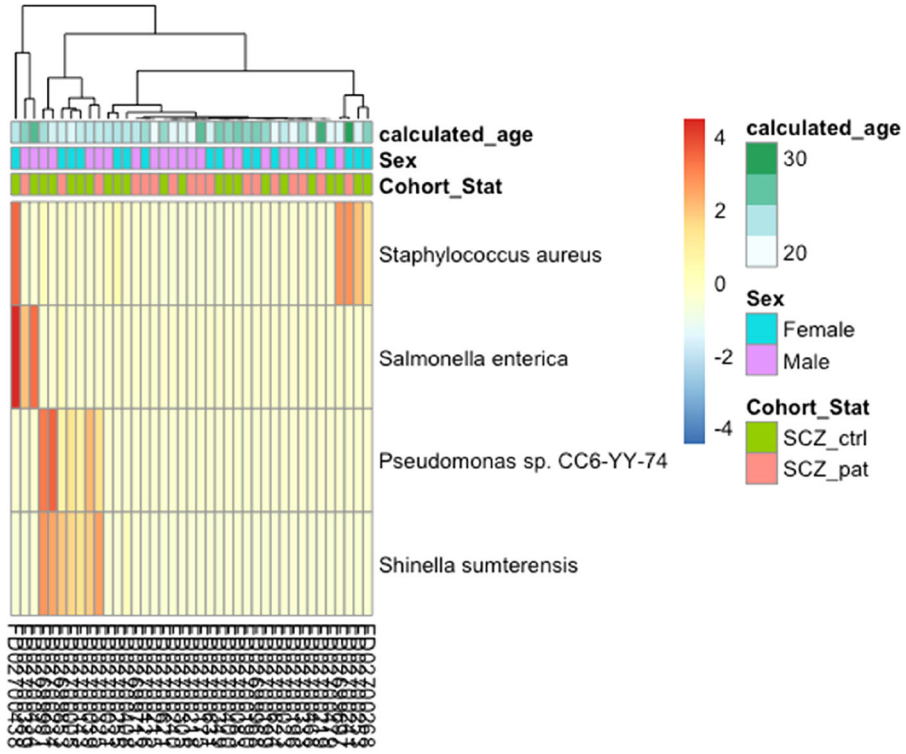

FIGURE 3 | Heatmap for statistically significant altered signature in SCZ cases compared to controls. The heatmap shows an enriched signature for *Salmonella enterica* and a statistically significant depleted signature for *Staphylococcus aureus*, *Pseudomonas* sp. CC6-YY-74, and *Shinella sumterensis* for SCZ cases compared to controls. The heatmap indicates in which samples the taxa abundance was enriched or depleted. Moreover, it indicates whether samples were female or male, and their age. No clear clustering of differential taxa expression was observed due to age or sex.

correction for multiple testing (Rust, Asmal, et al. 2025) and was not observed in the blood microbiome of the SCZ cohort.

4 | Discussion

In this study, we evaluated differences in the blood microbiome signatures between cases and controls in three neuropsychiatric disorders (PD, SCZ, and PTSD) by repurposing RNA-seq reads that did not map to the human genome, whereafter taxa signatures were correlated between the blood and the gut microbiome of the same cohorts.

4.1 | Blood Microbial Signatures

We observed an enriched abundance of *A. wuhouensis* and a depleted abundance of *P. aeruginosa* in the blood microbiome of PD cases versus controls. The genera *Acinetobacter* is regarded as an opportunistic human pathogen (Y. Hu et al. 2018) and has been reported in human femoral arteries (Sciarra et al. 2023). However, it should be noted that members of *Acinetobacter* genera found in water and soil environments have been observed as contaminating sequences in sequence-based microbiome analyses (Salter et al. 2014). The *Acinetobacter* species observed in our study, *A. wuhouensis*, was originally isolated from hospital sewage in Wuhou District, China, in 2015 (Y. Hu et al. 2018). Therefore, we cannot conclusively rule out the presence of *A. wuhouensis* being due to contamination as the taxa only represented less than 0.0% of total unmapped reads.

P. aeruginosa, another opportunistic pathogen (Lichtenberg et al. 2022), has been reported in a wide range of niches including the human microbiome and human femoral arteries (Sciarra et al. 2023). Furthermore, *P. aeruginosa* has been associated with acute and chronic infections including in the human respiratory tract (Lichtenberg et al. 2022). The presence of *Pseudomonas* has also been reported to be due to contamination from *Pseudomonas* spp. found in water and soil (Salter et al. 2014). Therefore, as *P. aeruginosa* represented less than 0.0% of total unmapped reads, we cannot exclude the possibility of the taxa signature being due to contamination. As all control participants compared to a single PD case had a blood microbial signature for *P. aeruginosa*, it may suggest a role for the taxa in individuals without PD. The colonization of *P. aeruginosa* in the respiratory tract has been reported in individuals who regularly inhale cigarette smoke (Chien et al. 2020). As individuals with PD are less likely to have smoked, we speculate that the lack of *P. aeruginosa* in PD cases compared to controls may be due to smoking habits.

In SCZ cases compared to the control group, the abundance of *S. enterica* was enriched, while the abundance of *S. aureus*, *Pseudomonas* sp. CC6-YY-74, and *S. sumterensis* were depleted. *S. enterica* is an opportunistic and foodborne pathogen and is considered a source of salmonellosis due to contaminated foods (Aljahdali et al. 2020). Moreover, *Salmonella* has been reported to induce intestinal inflammatory responses in humans (Aljahdali et al. 2020). As *Salmonella* is a pathogen observed in humans, the presence of this taxa signature in the blood microbiome may be expected. Furthermore, the intestinal inflammatory responses induced by *Salmonella* could possibly contribute to increased

TABLE 2 | Demographics and description of cohorts.

Status	Case *N* (%)	Control *N* (%)	*p*-value
PD ($n = 34$)	15 (44.1)	19 (55.9)	0.164[t]
SCZ ($n = 39$)	17 (43.6)	22 (56.4)	0.127[t]
PTSD ($n = 79$)	45 (57)	34 (43.03)	0.961[t]
Sex (Female)			
PD	8 (53.3)	7 (36.8)	0.254[c]
SCZ	8 (47.1)	10 (45.5)	0.922[c]
PTSD	38 (84.4)	27 (79.4)	0.374[c]
Age (years)	Case mean (SD)	Control mean (SD)	
PD	63.237 (7.474)	61.931 (6.452)	0.604[t]
SCZ	24.146 (4.939)	23.463 (3.241)	0.625[t]
PTSD	43.796 (https://doi.org/10.118)	45.939 (9.491)	0.337[t]

Abbreviations: N, number; PD, Parkinson's disease; PTSD, posttraumatic stress disorder; SCZ, schizophrenia; SD, standard deviation.
*$p < 0.05$; [c]chi-square test; [t]*t*-test.

TABLE 3 | Direction of significant bacterial signatures associated with the blood microbiome of three cohorts.

Taxa (species)	$\log_2$ fold change	Change	*p*-value	*q*-value (BH)
PD				
Mycobacterium sp. Z3061	0.549	↑	0.009	0.179
Pseudomonas aeruginosa	−26.647	↓	< 0.000	< 0.000*
Clostridium baratii	6.498	↑	0.022	0.300
Vibrio mediterranei	6.438	↑	0.030	0.300
Acinetobacter wuhouensis	21.899	↑	< 0.000	< 0.000*
Laribacter hongkongensis	6.161	↑	0.037	0.325
Serratia grimesii	6.446	↑	0.029	0.300
SCZ				
Staphylococcus aureus	−2.990	↓	< 0.000	< 0.000*
Salmonella enterica	25.494	↑	< 0.000	< 0.000*
Pseudomonas sp. CC6-YY-74	−24.416	↓	< 0.000	< 0.000*
Shinella sumterensis	−8.063	↓	0.002	0.033*

*$p < 0.05$ and $q < 0.05$.

TABLE 4 | Assessing the correlation of eight overlapping taxa between blood and gut microbiome using Procrustes analysis.

Overlapping taxa	PD			SCZ			PTSD		
	Case	Control	*p*-value	Case	Control	*p*-value	Case	Control	*p*-value
Clostridium	64.3	73.7	0.596	70.6	81.8	0.413	77.8	70.6	0.144
Streptococcus	64.3	52.6	0.473	52.9	54.5	0.187	20.0	8.8	0.397
Lactobacillus	14.3	0.0	0.248	0.0	0.0	NB	0.0	0.0	NB
Bacteroides	7.1	15.8	0.700	17.6	0.0	0.242	0.0	0.0	NB
Prevotella	0.0	0.0	NB	0.0	0.0	NB	97.8	100.0	0.771
Enterococcus	7.1	https://doi.org/10.5	NG	11.8	18.2	0.198	0.0	0.0	NB
Fusobacterium	0.0	0.0	NB	5.9	0.0	0.741	0.0	0.0	NB
Campylobacter	0.0	0.0	NB	5.9	0.0	0.036*	0.0	8.8	0.726

Note: Percentage represents number of participants with data for taxa.
Abbreviations: NB, not present in blood; NG, not present in gut.
*$p < 0.05$.

intestinal permeability to allow the translocation of microbial products, pathogens and inflammatory mediators into the blood. As *S. enterica* was only present in the blood microbiome of four participants (10.5%) in the SCZ cohort, it is likely due to contamination.

S. aureus colonization is considered harmless but a risk factor for developing acute or chronic infections such as septic arthritis, septicaemia, and pneumonia (Howden et al. 2023). Therefore, *S. aureus* is regarded as an opportunistic human pathogen (Howden et al. 2023). *Staphylococcus* spp. is a common genus in human blood samples and has been reported to be significantly elevated in inflammatory bowel disease and other various inflammatory disorders, as well as *Pseudomonas* (Cheng et al. 2023). Moreover, *Staphylococcus* has been associated with biological actions including oxidative phosphorylation in circulating cells (Cheng et al. 2023). Therefore, the observation of this taxon in the blood microbiome may be expected and not due to contamination. This is further supported by all participants in the SCZ cohort having blood microbial signatures for *S. aureus*.

However, *S. sumterensis* has not been reported in the human blood microbiome to date and is commonly associated with environmental samples, particularly soil and water (Arya et al. 2022). Furthermore, *S. sumterensis* represented less than 0.0% of total unmapped reads. This suggested that the presence of *S. sumterensis* in the blood microbiome may be due to contamination. This is further supported by only seven participants (17.9%) possessing blood microbial signatures for *S. sumterensis*.

No significant abundance differences in blood microbial signatures were observed in PTSD cases versus controls. Moreover, none of the significant abundance differences in blood microbiome signatures correlated with the gut microbiome taxa associated with the case-control status of PD, SCZ, or PTSD.

4.2 | Blood and Gut Microbial Correlation

We identified eight taxa present in both the gut and blood microbial data including *Clostridium* (PD, SCZ, PTSD), *Streptococcus* (PD, SCZ, PTSD), *Lactobacillus* (PD), *Bacteroides* (PD, SCZ), *Fusobacterium* (SCZ), *Enterococcus* (SCZ), *Campylobacter* (SCZ, PTSD), and *Prevotella* (PTSD). These results should be interpreted cautiously as the blood microbial signatures may have been biased due to low concentrations of microbial signatures (very low read count) that result in large apparent fold changes. The abundance of *Clostridium* has been previously associated with the case-control status of PD in the gut microbiome in our cohort. However, the abundance of *Clostridium* in the blood and gut microbiome of our PD cohort was not correlated.

Of the overlapping taxa in both the gut and blood microbiome of PD, SCZ, and PTSD in our cohorts, *Prevotella* (Jagare et al. 2023), *Lactobacillus* (Sciarra et al. 2023; Tan et al. 2023), and *Fusobacterium* (Tan et al. 2023) have been observed in the human gut microbiome and oral microbiome, while *Bacteroides* is commonly reported in the human gut microbiome (Sciarra et al. 2023). *Prevotella* and *Bacteroides* have been observed to be more abundant in the blood microbiome of individuals with respiratory diseases such as asthma (Cheng et al. 2023).

Streptococcus is a common skin (Salter et al. 2014) and oral taxa (Suparan et al. 2022) that have been reported to utilize red blood cells to evade innate immunity (Sciarra et al. 2023). In smoking individuals, *Streptococcus* have been associated with increased dyspnea severity (Cheng et al. 2023). Notably, *Streptococcus* has also been reported as a contaminant found in blank controls (Salter et al. 2014). Like *Staphylococcus*, *Streptococcus* and *Lactobacillus* have also been associated with oxidative phosphorylation in circulating cells (Cheng et al. 2023). Both *Streptococcus* and *Lactobacillus* have been observed in irritable bowel disease, characterized by increased intestinal permeability, which could allow translocation of microbial products to the blood (Cheng et al. 2023). A study on alcohol dependence in males found *Prevotella*, *Streptococcus*, *Fusobacterium*, and *Bacteroides* to be correlated between the oral and gut microbiome (L. Hu et al. 2023). *Clostridium*, reported in the gut microbiome, has also been found to be dominant in plasma and erythrocyte fractions in human blood (Castillo et al. 2019). *Enterococcus*, reported in the human gut microbiome, has been associated with translocation into the blood that could result in bacteremia (Dubin and Pamer 2017). *Campylobacter* is also considered an infectious pathogen in humans with infections often occurring in young adults (Aljahdali et al. 2020). *Campylobacter* is further suggested to predict overactivity of inflammatory responses and has been correlated with respiratory infection severity in cases such as COVID-19 (Cheng et al. 2023).

Therefore, the observation of these taxa in both the gut and blood microbiome of the respective PD, SCZ, and PTSD cohorts is expected due to reports regarding their presence in blood. Of note, *Lactobacillus* was only present in the blood microbiome of PD cases and not controls, similar to *Bacteroides*, *Fusobacterium*, and *Campylobacter*, which were only present in SCZ cases. Only two PD participants (14.3%) had microbial signatures for *Lactobacillus* in the blood microbiome. Three SCZ participants (17.6%) had microbial signatures for *Bacteroides* and only a single SCZ participant (5.9%) had microbial signatures for *Fusobacterium* and *Campylobacter* in the blood microbiome. Therefore, the observations are most likely due to contamination or individual-specific factors such as diet, lifestyle, comorbidities (e.g., constipation, periodontitis), and medication affecting mucosal permeability in the gut or mouth.

4.3 | RNA-seq Versus 16S rRNA

To date, the blood microbiome has not been extensively studied but has been reported on in several disorders including PD, SCZ, depression, IBS, and diabetes (Amar et al. 2013; Loohuis et al. 2018; Qian et al. 2018; Castillo et al. 2019; Whittle et al. 2019; Ciocan et al. 2021; Suparan et al. 2022; Cheng et al. 2023; Jagare et al. 2023; Sciarra et al. 2023). The majority of these studies investigated the blood microbiome through the use of PCR or qPCR to generate 16S rRNA gene sequencing data from blood samples, whereas only two reported the use of human RNA-seq data (Loohuis et al. 2018; Jagare et al. 2023). Furthermore, we used 16S rRNA (V4) gene sequencing to generate the gut microbiome data that were correlated with the blood microbiome in our study. This could constrain the comparison of our results based on RNA-seq data to previous studies, and gut microbiome data making use of 16S rRNA gene sequencing data as 16S rRNA sequencing data, as reported by other studies, have several limitations briefly discussed here.

The use of 16S rRNA gene sequencing to investigate the blood microbiome is limited by biases, incomplete taxa classification, and the inability to determine absolute microbial abundance (Nearing et al. 2021; Filardo et al. 2024; Matchado et al. 2024). Furthermore, blood microbiome studies face challenges due to sample size, sampling methods, and high human genetic background, which can inhibit downstream analysis (Reinicke et al. 2024; Wang et al. 2024). The majority of studies report the possibility of contamination that may have influenced results as the microbial signatures detected are considered low biomass or low read counts. Contamination could be introduced during sample collection (e.g., from the skin, venipuncture), during processing due to bacterial and free DNA, and during PCR preparation due to cross-contamination and residual foreign DNA that may reside in PCR reaction compounds (Nearing et al. 2021; Reinicke et al. 2024). The influence of these contaminants can be prevented by mitigating external contaminants and measuring the degree of internal contamination by the use of appropriate negative and positive controls in all steps of the pipeline (Poore et al. 2020). Internal contamination can be difficult to identify without knowledge regarding other samples that were run at the same time. Ideally, three types of negative control samples should be used, including DNA extraction blanks, DNA library preparation blanks, and empty control wells (water controls) (Poore et al. 2020). The lack of controls to address these possible origins of contamination is a current hindrance to blood microbiome studies, as it is difficult to therefore account for false positives (Chen et al. 2024) and distinguish between commensal species and those causing bloodstream infections or contamination (Reinicke et al. 2024).

Current available studies rarely describe the decontamination practices employed, and some attempts may not be stringent enough (Cheng et al. 2023). Moreover, there are currently no golden standards to ensure contamination-free blood microbiome results and innovative decontamination practices are needed post-sequencing to validate and ensure true representations of taxa present in the blood (Cheng et al. 2023). RNA-seq might offer a potential advantage of avoiding contamination of genomic DNA by dead cells in the blood compared to 16S rRNA genome sequencing, as RNA-seq data represent all the expressed RNA in the sample. However, RNA-seq can still be affected by external (e.g., reagents, environmental factors, sampling errors) and internal (e.g., cross-contamination) contamination that should be identified and considered when investigating the blood microbiome based on RNA-seq data (Poore et al. 2020). Whittle et al. (2019) minimized external contaminants during plasma sampling for RNA-seq by cleaning the skin with alcohol prior to drawing blood.

Moreover, RNA-seq data could provide functional insight that targeted 16S rRNA gene sequencing data lack. The use of 16s rRNA gene sequencing for blood and gut microbiome studies may also introduce biases in gene and function inference possibly due to functional redundancy and poor accuracy at the species level (Cheng et al. 2023). For gut microbiome data, 16S rRNA gene sequencing is standard practice, although a lack of species-level data can lead to misinterpretation of results as species within the

same genus can have different functional roles and may also be a limitation in blood microbiome studies (Ranjan et al. 2016).

The variation in current blood microbiome results also suggests that a core blood microbiome may not contribute to host functionality as the blood niche may not be suitable for prolonged colonization and only function as an intermediate niche during translocation between other niches (Cheng et al. 2023). This may be associated with increased microbial translocation suggested to contribute to the blood microbiome due to decreased mucosal integrity (in the gut, brain, and oral) observed in several disease states including PD, SCZ, PTSD, and depression (Cheng et al. 2023). Furthermore, differences in blood microbiome reports could also be influenced by sample type as different bacterial diversities have been reported in red blood cells, plasma, and whole blood (Cheng et al. 2023).

4.4 | Limitations

This study was performed retrospectively, which contributed to limitations in our study. Our study could have potential contamination (during phlebotomy, and background contamination from DNA extraction and reagents), known to impact the analysis of low-biomass samples, as the samples were not originally intended to be used for analysis of microbial sequences and therefore lack controls that should be included for contamination. We did not attempt diversity metrics, as the tests are sensitive to biological and technical variability, and in the low-powered settings, may yield spurious or non-reproducible differences. In contrast, DESeq2 is specifically designed for sparse count data and incorporates shrinkage estimation, which makes it more suitable for detecting robust, taxa-specific differences even in small cohorts. Therefore, we prioritized differential abundance testing.

Furthermore, our study lacked validation of blood microbiome results, which is important to confirm the true profile of the blood microbiome and exclude possible contaminating taxa signatures. Validation could be achieved through culture-dependent or culture-independent methods, although culture-dependent profiling may be hindered by fastidious and non-culturable microbial taxa. Therefore, future studies should consider culture-independent methods to validate blood microbial results in an attempt to determine if the RNA extracted was from a viable organism or cell-free nucleic acids of dead pathogens due to recent infections.

The correlation between the gut and blood microbiome could have been hindered by a small overlapping sample size (PD case: 3, control: 11; SCZ case: 10, control: 19; PTSD case: 26, control: 9). Furthermore, we lacked information on other human microbiome niches such as the skin and oral microbiome to evaluate possible translocation from these areas to the blood.

5 | Conclusion

Our study overcomes several of the limitations posed by 16S rRNA gene sequencing by making use of RNA-seq data. The use of RNA-seq in our study does suggest that recent microbial activity produced an RNA product; however, it does not prove that the RNA extracted was from a viable organism or cell-free nucleic acids of dead pathogens due to recent infections (Cheng et al. 2023). All the reported taxa identified in this study have been reported in human blood samples, except *S. sumterensis*, and therefore could suggest that our observations were not due to external contamination. However, our results do not necessarily support the hypothesis that these taxa are translocated from the gut, as several of the taxa are also observed in the oral microbiome. Further research is required to understand the biological implications of these findings, in particular, taking into account the repurposed data source, and the potential for contamination (during phlebotomy, and background contamination from DNA extraction and reagents) known to impact the analysis of low-biomass samples.

Author Contributions

C. Rust: investigation, writing - original draft, methodology, visualization, formal analysis. **D. Tonge**: writing - review and editing, methodology. **L. L. van den Heuvel**: writing - review and editing, data curation. **L. Asmal**: writing - review and editing. **J. Carr**: writing - review and editing. **E. Pretorius**: conceptualization, writing - review and editing, supervision. **S. Seedat**: funding acquisition, writing - review and editing, supervision. **S. M. J. Hemmings**: conceptualization, writing - review and editing, supervision.

Acknowledgments

C.R. is supported by the Stellenbosch University Postgraduate Scholarship Programme. C.R., S.S., and S.M.J.H are supported by the South African Medical Research Council (SAMRC)/Stellenbosch University Genomics of Brain Disorders (GBD) Extramural Unit. Research reported in this study was supported by the South African Medical Research Council for the "Shared Roots" Flagship Project, Grant MRC-RFA-IFSP-01-2013/SHARED ROOTS through funding received from the South African National Treasury under its Economic Competitiveness and Support Package. The work by L.L.v.d.H was supported in part by the National Research Foundation of South Africa (Grant 138430) and by the South African Medical Research Council under a Self-Initiated Research Grant. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the South African Medical Research Council. During the preparation of this work, the first author(s) used ChatGPT in order to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Ethics Statement

This study is a sub-study of the project entitled "Understanding the SHARED ROOTS of Neuropsychiatric Disorders and modifiable risk factors of cardiovascular disease" (ethics number: S22/05/094 [PhD] sub-study N13/08/115), which was approved by the Stellenbosch University Health Research Ethics Committee 1 (HREC1). The patients/participants provided their written informed consent to participate in this study.

Consent

Written informed consent was obtained from participants in order to perform clinical assessments, metabolic syndrome screening, neurocognitive tests, and genomic analyses. Male and female individuals over the age of 18 years old were eligible to participate. Demographic information, including age, sex, level of education, and personal and family medical history, was collected from all participants. Participants recruited for the primary flagship project were of the mixed ancestry population (self-identified) in South Africa.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets presented in this article are not readily available due to ethical and legal restrictions. Requests to access the datasets should be directed to the corresponding author. The authors are open to collaborating and sharing data within the limits of ethical review restrictions and data transfer policies of Stellenbosch University.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/brb3.70629

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Table 1:Number of unmapped reads per sample in the four major domains for each cohort. **Supporting Table 2**:Fold- change of blood microbiome species found in each cohort Parkinson's disease (PD).