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Original Article

Comparison of the prokaryotic and eukaryotic microbial communities in peripheral blood from amyotrophic lateral sclerosis, multiple sclerosis, and control populations

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A B S T R A C T

Neurodegenerative diseases are estimated to afflict hundreds of thousands of Americans with vastly more worldwide. The etiologies of amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) have yet to be established. Previous studies have suggested an association of these diseases with viruses, bacteria, and eukaryotic microbes, no new therapies have been forthcoming. High-throughput DNA sequencing has enabled the comprehensive analysis of microbial DNA profiles in diseased populations. To date, no amplicon-based next-generation DNA sequencing prokaryotic and eukaryotic community profiling studies have been completed for these diseases. Analysis of peripheral blood samples from control participants as well as ALS and MS participants was used to characterize the hematologic population of microbial DNA. Categorical and multivariate analysis with control for multiple comparisons and aged matched controls revealed differences in microbial DNA contribution in ALS patients compared to others. Notably, sequences that belonging to *Ochrophyta* were enriched in ALS patient samples. Mechanisms underlying this association, the role of microbial DNA sequences, and the development or progression of ALS may become a fertile subject of inquiry.

1. Introduction

Amyotrophic lateral sclerosis (ALS) and forms of multiple sclerosis (MS) are devastating progressive neurodegenerative diseases. ALS typically presents later in life and usually results in fatal paralysis within 1–5 years post-diagnosis [1,2]. MS is less severe, but results in an earlier average mortality often related to subsequent long-term disabilities [3]. The incidence of ALS is approximately 1.2–2.7 per 100,000 person-years, whereas the incidence of MS is higher at 2.0–3.6 per 100,000 person-years [4–6]. Overall, ALS and MS afflict approximately 12,000 and 400,000 people in the United States, respectively [7–9]. Despite extensive research, ALS and MS do not have well-established triggering etiologies. ALS research has revealed genetic risk factors; however, some familial- and most sporadic-cases are not explained by these mechanisms [2,10–14].

It has been proposed that ALS proceeds by a multi-step process in which a predisposition may be triggered by an event, such as infection [15]. A viral etiology in ALS has been considered, with focus on enteroviruses [16], such as polioviruses and echoviruses [17–19], retroviruses, including HIV [20], HTLV [21], and endogenous retroviruses [22]. Patients with HIV can exhibit ALS-like symptoms [23–25] and elevated reverse transcriptase activity has been detected in ALS patients [26,27]; however, anti-retroviral therapy has not been efficacious [28]. Furthermore, the role of bacteria in ALS has been examined, targeting *Mycoplasma* species [29] and *Borrelia burgdorferi* [30,31]. These relationships remain controversial [31] and have not established a treatment paradigm. Additionally, ALS epidemiologic factors suggests a role for cyanobacteria [32].

The involvement of eukaryotic infectious agents in MS has been investigated, i.e. *Plasmodium* species [33]. Interestingly, MS prevalence appears to be linked geographically and temporally to the seasonal activity of ticks and possibly other disease vectors [34]. Furthermore, treatment with doxycycline and minocycline, an anti-malarial therapeutic [35,36], can be effective in treating MS [37,38]. However, this effect may be due to reductions in systemic inflammation [39].

Eukaryotic microbes, including protozoa and fungi, are known to infect the nervous system [40–43]. Infection by *Toxoplasma gondii* is a risk factor for Parkinson's and Alzheimer's disease [44,45]. Agnostic identification of microbial DNA in ALS, MS, and unaffected individuals may uncover new microbial mechanisms in neurological illnesses. Next-generation DNA sequencing (NGS) can effectively profile microbial communities, including novel or unexpected organisms. Previous applications of this methodology include the analysis of atheroma debris and arterial filters, as well as the study of microbial DNA sequences in

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myalgic encephalitis, or chronic fatigue syndrome [46,47]. Likewise, this study utilizes direct-from-sample 16S and 18S rRNA sequence community analysis of peripheral blood from patients with MS, ALS, and from a control population.

2. Methods

2.1. Patient recruitment and selection

All participants with ALS met the El Escorial and Awaji criteria and were evaluated with the ALS Severity Score (ALSSS) [48–50]. All participants with MS met the revised McDonald criteria and were evaluated with the Expanded Disability Severity Score (EDSS) and with Multiple Sclerosis Severity Score (MSSS) [51–53]. Control participants were self-reported as healthy and did not suffer from a debilitating illness at the time of recruitment. Participants, between 18 and 90 years of age, were recruited by their primary care physician in the Phoenix, Arizona metropolitan area, provided written informed consent, and provided the blood sample during a routine scheduled outpatient clinical visit. The study design and recruitment were approved by the Western Independent Review Board (WIRB; Protocol #21211127, Study #1133561). This research was conducted in accordance with the Declaration of Helsinki.

2.2. Sample collection and DNA isolation

Peripheral blood samples were collected from patients were collected by standard venipuncture from the median cubital vein (BD Vacutainer K2 EDTA, ~3 mL) and were transported with refrigeration via rapid courier. Total DNA from peripheral blood was extracted from 200 μL (QIAamp DNA Blood Mini Kit, Qiagen) with 10 min vortex agitation (50 μL 1 mm silicate beads, Next Advance) and eluted into 30 μL of AE buffer, as previously described [47].

2.3. Next-Generation DNA sequencing and microbial identification

Fusion primers and low stringency PCR was used to generate sequence ready amplicons [47]. As previously described, the resulting amplicons were size-selected (PippinBlue, Sage Science), pooled in equimolar concentration (Qubit dsDNA HS Kit, ThermoFisher), and sequenced (Ion Torrent Personal Genome Machine, LifeTechnologies) [47]. The raw sequence FASTQs were processed using the Rapid Infectious Disease Identification (RIDI™) system (U.S. Patents 9,971,867, 9,971,979, and 9,589,101, Fry Laboratories, LLC) [47,54]. To allow for the detection of potential novel organisms, a minimum of 80% percent identity was used. To be included in the analysis, a sequence was at least 75 bp or 60 bp for the prokaryotic and eukaryotic results, respectively. On average the identified reads were 96.18% ± 3.35% and 99.31% ± 1.85% identical to the reference eukaryote or prokaryote reference, respectively. Genomic data was deposited in the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) (PRJNA422103, control samples; PRJNA485941, ALS and MS samples).

2.4. Statistical analyses

Multivariate statistical analysis was performed using Analyse-It v4.80 (Analyse-It Software, Ltd, Leeds, UK). The violin plots and quartiles were generated using Matplotlib and Numpy (Python 2.7.11, matplotlib 2.0.2, numpy 1.13.1). Chi-squared, Bonferroni, Holm, Benjamini-Hochberg, and Cohen's d statistical analysis were performed using Excel (Microsoft). All Welch's t-tests were performed using the stats.ttest_ind function with unequal variance (Python 2.7.11, Scipy 0.17.0).

2.5. Contamination and artifact control

Artifact and sample contamination were controlled as previously described by our laboratory [46,47]. Contamination risk points include sample collection, DNA extraction, and molecular amplification. Sequencer induced contamination is minimal as the presence of adapter and barcode sequences are unlikely to be present in unintended sequences. Run-to-run contamination was insignificant as no unintended barcodes were recovered. Sequencer-introduced artifact reads, homopolymers, non-barcoded sequence reads, and floworder artifacts were removed from analysis. Standard sterile venipuncture collection methods, alcohol skin swabbing, were implemented to reduce collection-based artifacts such as skin microbiota. Collection tube contamination was controlled as all collection tubes were from the same manufacturing lot and were randomized when provided to each collection site. PCR contamination was controlled by UV-sterilization and a dedicated PCR room. Co-prepared negative controls, that shared a master mix, controlled for reagent contamination and did not yield the appropriate size or measurable amounts of amplicons that could be sequenced.

3. Results

The average age of the MS and ALS participants was statistically older when compared to the control participant population, as expected. Also, the MS population exhibited an enrichment for female participants, consistent with previous studies [55]. Likewise, the enrolled ALS population was marginally enriched with male participants, consistent with epidemiologic studies [8,9], but not statistically significant. The ALS and MS severity scores were 24.07 ± 6.69 and 3.20 ± 4.36, respectively (Table 1).

Eukaryotic and prokaryotic DNA reads observed in at least 2 samples, consisting of at least 10 independent reads, and at least 5% of the population were compiled. A total of 7 eukaryotic and 27 prokaryotic genera met this criterion. Observations were compiled for higher taxonomic levels including 2 eukaryotic phyla and a single eukaryotic superphylum, in addition to 3 prokaryotic phyla and 3 prokaryotic classes (Table 2).

The MS sample DNA sequences do not reveal a significant difference from the control samples when controlled for multiple comparisons. However, detection of the *Hydrurus* genus was significantly increased in ALS samples, with detection in all 15 ALS samples, but only in 11 of 44 control samples ($P < 0.00001$). Also, the phylum-level taxon containing the *Hydrurus* genus, *Ochrophyta*, was detected in all 15 ALS samples and in 19 of 44 control samples ($P = 0.00012$). The most

Table 1
Participant Demographics and Severity Scores.

	Participants (n)	Age (yrs)	Age *P*	Gender (M/F)	Gender *P*	ALSSS	MSSS
ALS	15	61.40 ± 10.54	< 0.001	60.0%(9)/40.0%(6)	0.414	24.07 ± 6.69	–
MS	32	53.84 ± 12.82	< 0.001	21.9%(7)/78.1%(25)	0.018	–	3.20 ± 4.36
Control	48	42.73 ± 13.95	–	47.9%(23)/52.1%(25)	–	–	–

ALS, amyotrophic lateral sclerosis; ALSSS, ALS severity score; M, male; F, female; MS, multiple sclerosis; MSSS, MS severity score.

Table 2
Detection of Genera and Higher-Order Classifications.

Closest Matching Genera		Observations	Class	% Contribution
Prokaryota	*Pseudomonas*	86	*γ-proteobacteria*	28.9% ± 17.9%
	Acinetobacter	58	*γ-proteobacteria*	4.5% ± 8.5%
	Acidovorax	50	*β-proteobacteria*	4.3% ± 6.2%
	Propionibacterium	42	*Actinobacteria*	2.8% ± 4.6%
	Paracoccus	39	*α-proteobacteria*	2.1% ± 3.1%
	Stenotrophomonas	37	*γ-proteobacteria*	1.7% ± 2.8%
	Pseudoxanthomonas	35	*γ-proteobacteria*	1.9% ± 3.6%
	Sphingomonas	34	*α-proteobacteria*	2.4% ± 5.5%
	Brevundimonas	32	*α-proteobacteria*	2.1% ± 3.8%
	Diaphorobacter	30	*β-proteobacteria*	1.7% ± 3.9%
	Flavobacterium	29	*Flavobacteriia*	1.2% ± 2.5%
	Sphingobium	28	*α-proteobacteria*	2.9% ± 6.4%
	Ralstonia	26	*β-proteobacteria*	4.9% ± 12.7%
	Cloacibacterium	23	*Flavobacteriia*	0.9% ± 2.0%
	Corynebacterium	21	*Actinobacteria*	0.9% ± 1.9%
	Phenylobacterium	19	*α-proteobacteria*	0.6% ± 1.5%
	Comamonas	18	*β-proteobacteria*	1.1% ± 2.9%
	Staphylococcus	18	*Bacilli*	0.7% ± 1.7%
	Neisseria	17	*β-proteobacteria*	2.1% ± 6.2%
	Streptococcus	17	*Bacilii*	0.9% ± 2.7%
	Agrobacterium	15	*α-proteobacteria*	0.8% ± 2.0%
	Pseudorhodoferax	15	*β-proteobacteria*	0.7% ± 1.9%
	Janthinobacterium	13	*β-proteobacteria*	0.4% ± 1.1%
	Lactobacillus	11	*Bacilli*	0.6% ± 2.9%
	Clostridium	12	*Clostridia*	0.6% ± 1.9%
	Aquabacterium	10	*β-proteobacteria*	0.4% ± 1.4%
	Pedobacter	10	*Sphingobacteriia*	0.3% ± 0.9%
Eukaryota	*Funneliformis*	73	*Glomeromycetes*	60.9% ± 43.0%
	Hydrurus	34	*Chrysophyceae*	21.7% ± 34.7%
	Spumella	18	*Chrysophyceae*	5.1% ± 15.1%
	Ochromonas	17	*Chrysophyceae*	3.1% ± 8.8%
	Chrysonebula	12	*Chrysophyceae*	0.3% ± 0.7%
	Kephyrion	11	*Chrysophyceae*	0.3% ± 1.1%
	Perkinsus	11	*Perkinseae*	4.1% ± 16.5%
Groups		Observations	Taxonomy Rank	% Contribution
Prokaryota	*γ-proteobacteria*	86	*Class*	36.9% ± 21.7%
	β-proteobacteria	75	*Class*	15.8% ± 16.7%
	α-proteobacteria	68	*Class*	11.1% ± 11.5%
	Actinobacteria	46	*Phylum*	3.8% ± 5.7%
	Bacteroidetes	46	*Phylum*	2.5% ± 3.3%
	Firmicutes	43	*Phylum*	2.8% ± 4.5%
Eukaryota	*Opisthokonta*	73	*Phylum*	60.9% ± 43.0%
	Ochrophyta	49	*Phylum*	30.5% ± 38.1%
	Alveolata	11	*Superphylum*	4.1% ± 16.5%

Table 3
Comparison of Categorical Observations.

	ALS		Control		Control for Multiple Comparisons			
Observations (Taxa)	Detected	Not Detected	Detected	Not Detected	*P*	Bonferroni	Holm	Benjamini-Hochberg
Funneliformis (Genus)	0	15	41	3	< 0.00001	0.00029	0.00029	0.00029
Hydrurus (Genus)	15	0	11	33	< 0.00001	0.00029	0.00030	0.00059
Ochrophyta (Phylum)	15	0	19	25	0.00012	0.00360	0.00360	0.00360

significant result difference between ALS and control samples is a genus of mycorrhizal fungi, *Funneliformis*, which was absent in the ALS samples and present in 41 of the 44 control samples ($P < 0.00001$). Approximately, a single significant association is predicted out of the 34 genera and 9 higher-order taxa observed (0.41 precisely, at $P = 0.01$). However, 3 observations were statistically significant, consistent with a true difference (Table 3). Additionally, the displacement of *Funneliformis* in the ALS samples and the agreement between *Hydrurus* and its phylum is consistent with the sample identity rather than a result of chance association.

The categorical results suggest that several taxa are found in both patient samples and control samples. The differences in the relative contribution from each of these taxa to the total sequence results could be used to detect additional associations or confirm the categorical results. Relative contribution was calculated as the percentage of sequences for each of the 43 genera and higher-level taxonomic categories. This relative contribution was compared across the three sample groups. No statistically significant differences were observed between MS and control samples and the observed microbial DNA profiles were similar (Fig. 1). However, significant differences between ALS and both MS and control samples were observed for several genera and higher taxa (Fig. 1, Table 4).

Based on random chance alone, the relative contribution of approximately 1 of the detected genera is expected to be statistically

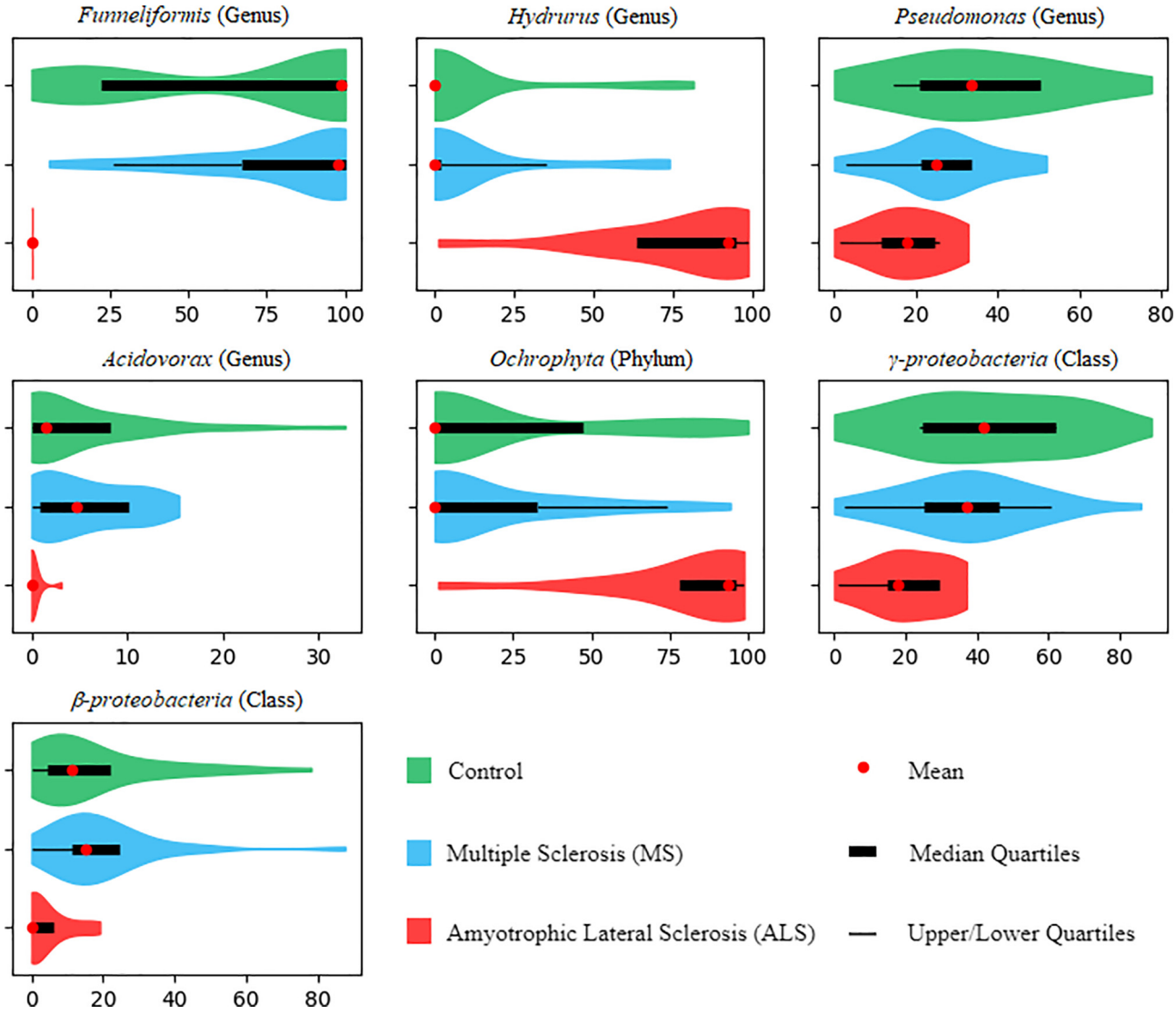

Fig. 1. Violin plots of the statistically significant results. The percent contribution of the sequence results illustrates positive (*Hydrurus* and *Ochrophyta*) and negative (*Funneliformis*, *Pseudomonas*, *Acidovorax*, γ-*proteobacteria*, and β-*proteobacteria*) associations with ALS (red) as compared to results from control (green) and MS samples (blue). Upper and lower quartiles (thin black lines), means (red circles), and median quartiles (thick black lines) are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

different between groups (0.34 precisely, at $P \leq 0.01$). The relative contributions of 6 genera were significantly different between groups ($P \leq 0.01$). As with categorical analysis, the *Hydrurus* genus was significantly enriched in ALS samples compared to controls, whereas *Funneliformis*, *Pseudomonas*, *Acidovorax*, *Acinetobacter*, and *Cloacibacterium* were significantly reduced in ALS samples (Table 4). The Bonferroni and Holm–Bonferroni methods were used to control for multiple comparisons, while false discovery was controlled by the

Table 4
Statistical Analysis of Detected Microbial Contribution Percentage.

Genus Level Observations	ALS (avg reads)	Control (avg reads)	Effect Size (Cohen's d)	Welch's *P*	Bonferroni	Holm	Benjamini–Hochberg
Funneliformis	0.0% ± 0.0% (0.0)	66.9% ± 41.4% (5301.3)	2.29	< **0.00001**	0.00029	0.0003	0.00029
Hydrurus	77.7% ± 27.2% (45440.7)	10.4% ± 24.1% (2839.3)	2.62	< **0.00001**	0.00029	0.0003	0.00059
Pseudomonas	17.4% ± 10.1% (754.9)	34.7% ± 20.9% (285.9)	1.06	**0.00009**	0.00029	0.0003	0.00088
Acidovorax	0.3% ± 0.8% (10.7)	4.9% ± 7.4% (39.8)	0.88	**0.00019**	0.00029	0.0003	0.00118
Acinetobacter	0.9% ± 2.2% (53.1)	4.5% ± 5.7% (35.9)	0.83	0.00102	0.00029	0.0003	0.00147
Cloacibacterium	0.0% ± 0.0% (0.0)	1.0% ± 2.0% (4.8)	0.71	0.00176	0.00029	0.0003	0.00176
Higher Taxa Observations	ALS (avg reads)	Control (avg reads)	Effect Size (Cohen's d)	Welch's *P*	Bonferroni	Holm	Benjamini–Hochberg
Ochrophyta (Phylum)	80.7% ± 26.9% (46795.5)	22.1% ± 34.9% (5768.5)	1.88	< **0.0001**	0.0036	0.0036	0.0036
γ-*proteobacteria* (Class)	20.5% ± 11.9% (900.7)	42.5% ± 23.5% (372.2)	1.18	< **0.0001**	0.0036	0.0038	0.0071
β-*proteobacteria* (Class)	4.1% ± 6.3% (180.7)	17.0% ± 18.3% (187.1)	0.95	**0.0002**	0.0036	0.0042	0.0107
Firmicutes (Phylum)	0.9% ± 2.1% (44.9)	3.5% ± 5.5% (22.5)	0.61	0.0135	0.0036	0.0045	0.0143

The bolded values meet or exceed significance when controlled for multiple comparisons.

Benjamini–Hochberg method. The effect size for the increased level of *Hydrurus* and the depletion of *Funneliformis* in ALS samples are both classified as "huge" in effect size. A single comparison (0.07 precisely, at $P \leq 0.01$) of the higher taxonomic measurements would be expected to be significantly different based on chance alone, but 2 phylum-level, and 2 class-level measurements exhibited statistical significance at $P \leq 0.01$ (*Ochrophyta*, *γ-proteobacteria*, *β-proteobacteria*, and *Firmicutes*). Controlling for multiple comparisons and the false discovery rate supported the significant differences in *Ochrophyta* and the *Proteobacteria* observations. The effect sizes for all higher taxonomic measurements were considered "large" by statistical standards (Table 4).

For organisms suggested to be associated with or potentiate ALS, it would be expected that detection of the organism would correlate with severity scores. In addition to categorical analysis, multivariate correlation analysis between the proportion of the detected DNA to the patient severity score could reveal roles for additional DNA sequence categories or confirm the categorical observations. Several DNA sequence groups displayed weak individual correlations, both positively and negatively, with the severity score (Pearson's r, Spearman's rs, and Kendall's tau; *data not shown*); however, categorical assignment using a subset of correlative metrics does result in useful clustering (Fig. 2, red ellipse). The relative contributions of *β-proteobacteria*, *γ-proteobacteria*, and *Ochrophyta* sort ALS patients from both the MS and control populations. These combined results represent a statistically significant category that differentiates the ALS samples from the MS and control samples (Table 5, $p < 0.001$).

Table 5
Categorical Analysis of Grouping by *Ochrophyta*, *β-proteobacteria*, and *γ-proteobacteria*.

Category	Clustered, % (n)	Nonclustered, % (n)	*P*
ALS	86.7% (13)	13.3% (2)	< **0.001**
Control	0.0% (0)	100.0% (44)	
ALS	86.7% (13)	13.3% (2)	< **0.001**
MS	3.1% (1)	96.9% (31)	
MS	3.1% (1)	96.9% (31)	0.238
Control	0.0% (0)	100.0% (44)	

Furthermore, to eliminate additional confounding variables, age-matched results were analyzed for the ALS and control participants. A total of 8 age-matched groups were generated for the overlapping

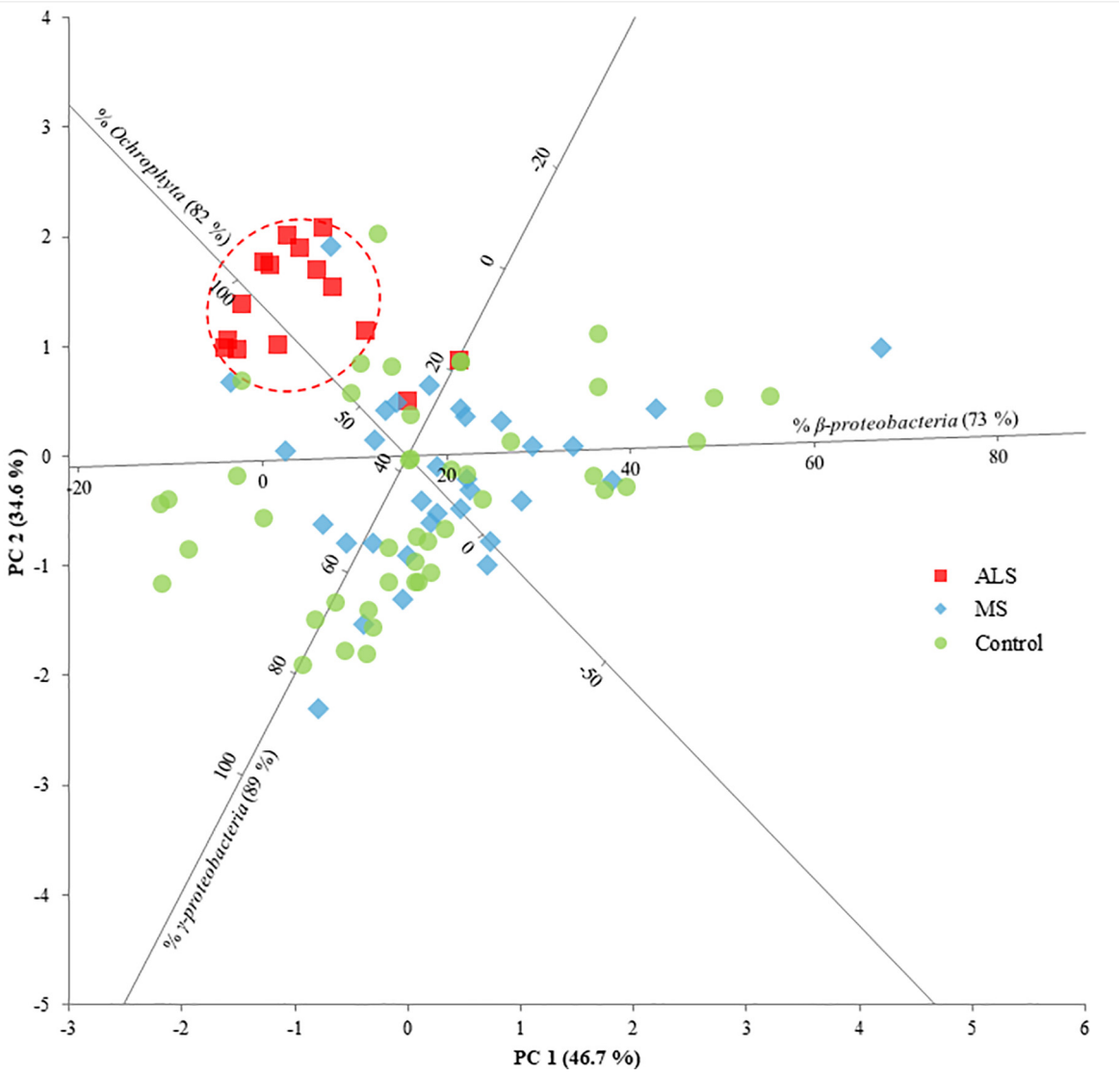

Fig. 2. Principal component analysis bi-plot. The plot represents 81.3% of variation observed in all three sample populations, with the PC1 and PC2 axis consist of 46.7% and 34.6% of the variation, respectively. Three organism categories (*Ochrophyta*, *β-proteobacteria*, and *γ-proteobacteria*) were found to effectively sort the ALS samples (red squares) into a cluster (dotted red ellipse) from both the MS (blue diamonds) and control (green circles) sample populations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Age-Matched Group Subanalysis.

Observations	ALS	Control	Welch's *P*
Age	54.88 ± 8.08	55.25 ± 8.33	0.92850
Group Size (n)	8	8	–
Funneliformis (Genus)	0.000 ± 0.000	49.70 ± 51.56	0.02950
Hydrurus (Genus)	87.58 ± 9.54	21.14 ± 36.57	0.00111
Ochrophyta (Phylum)	90.82 ± 6.70	32.28 ± 44.122	0.00697

ALS, amyotrophic lateral sclerosis.

patient age groups consisting of ALS and control participants (Table 6).

Age-matched groups were defined as a year or less difference in age. The results for years with multiple representative patients were generated as an average of the individual contributing patient results. As expected, no significant difference in age was detected ($p = 0.9285$). Consistent with differences observed from the entire participant pool, the previous top 2 genus-level and the top higher-taxonomic findings were confirmed as significant in the age-matched groups.

4. Discussion

In this study, microbial DNA sequences from blood obtained from ALS and MS participants were compared to sequences from control participant samples. The microbial DNA from MS samples was similar to sequences from control samples. In contrast, a unique microbial DNA signature was observed in ALS samples, but not control samples. DNA sequences best matching *Ochrophyta*, a phylum-level taxon comprised of heterokonts [56,57], were detected in all ALS samples, but in only 43.2% of control samples. DNA from the *Hydrurus* genus, freshwater algae within the *Ochrophyta* taxon [58], were detected in all ALS samples, but in only 25.0% of both the MS and control sample pools. The detection of *Hydrurus* species DNA in ALS samples potentially displaces other eukaryotes in MS and control samples. The enrichment of *Hydrurus* DNA in the ALS samples represents one of the most striking and unexpected findings of this study; therefore, we independently confirmed the presence of *Hydrurus* DNA in the ALS samples by using different primers that generated shifted and non-overlapping fragments that were verified by sequencing (Supplemental Fig. 1).

Funneliformis, *Pseudomonas*, and *Acidovorax* sequences were significantly depleted in ALS samples (Table 4). A hypothetical protective role for microbes in ALS is difficult to model; however, such a benefit cannot be excluded. Alternatively, the microbial DNA may represent a microbiota background; whereby, PCR competition may selectively suppress the background signal in ALS samples, while the background is indistinguishable in MS and control samples. Consistent with this explanation, organisms that are enriched in the MS and control samples, but not ALS samples, are cosmopolitan; *Acinetobacter* and *Acidovorax* species, common in soil and/or groundwater [59–61] and *Funneliformis* species, a ubiquitous mycorrhizal fungus [62,63]. The most significant inversely-correlated results have been previously detected in blood samples, namely *Acidovorax* and *Pseudomonas* species [64]. Recently, a study of cell free DNA from 1351 samples yielded more than 100 million non-human sequences; some were consistent with *Pseudomonas*, *Acidovorax*, and *Acinetobacter* species [65]. Overall, DNA from both prokaryotic and eukaryotic microbes were detected in all groups and may potentially be consistent with a previously considered hematologic human microbiome [64]. Further, studies have detected microbes at low levels in blood samples, some of which are not cultivatable by routine methods [66].

A potential explanation whereby microbial DNA is present in blood samples is that the organisms may be present within the circulatory system. *Hydrurus foetidus* is found in association with biofilms in flow environments [67,68], possibly consistent with vasculature colonization; however, confirmation *in situ* would be required. Reduced blood flow and early haemodynamic disturbances occur in the brain of ALS patients [69,70].

The neurotoxin β-methylamino-L-alanine (BMAA) and its role in neurological illnesses has gained attention [71]. BMAA and ALS-like illness were initially traced to cyanobacteria-contaminated food sources in Guam [72]. Furthermore, BMAA has been suggested to play a role in Gulf War Veterans Illness through desert algal mat dust [73]. Interestingly, BMAA has been detected in post-mortem samples from individuals with ALS and Alzheimer's disease [74]. These studies spurred a phase I clinical trial using L-serine to delay ALS progression [75]. Eukaryotes have been reported to also produce BMAA and other toxins, generally diatoms and dinoflagellates [76–79]. Several mechanisms could explain a role of *Hydrurus* species in ALS; including, colonization and subsequent exposure or consumption of neurotoxins, brain hematological flow disturbances, or immunological alterations due to exposure may represent some possible scenarios. Alternatively, microbial differences between diseases may present secondarily due to disease alterations of the innate or humoral immunity [80]. If an association is established, microbial DNA may eventually be useful to differentiate classes of neurological illnesses.

Future studies may also characterize both DNA and RNA viruses in these populations. However, previous shotgun sequencing-based approaches have suffered from reagent contamination that obscures microbial detection [81]. Significantly fewer eukaryotic microbes have been fully sequenced or described when compared to prokaryotes, thus limiting analysis. Additionally, sequence contamination cannot be conclusively ruled out as the microbes detected in ALS samples are not consistent with previously reported or expected contamination. The use of NGS increases the chance of false positive results; however, controlling for multiple comparisons suggests these results are significant.

5. Conclusions

In this study, NGS-based methods were used to profile microbial DNA in blood samples. This enabled analysis of the relationships between diseases and microbial DNA sequences. Detection of *Ochrophyta* DNA, and inversely γ-*proteobacteria* and β-*proteobacteria* DNA, correlated with ALS, but not MS. Furthermore, the microbial DNA in MS samples cannot be differentiated from the DNA patterns in control samples. The role that microbial DNA plays in ALS remains unresolved; however, it may be related to host susceptibility, host colonization competency, or environmental exposure. An etiologic relationship between these results and illnesses has yet to be established, while the detection of microbial DNA may eventually prove informative in the study, diagnosis, classification, or treatment of ALS and other neurological illnesses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humic.2019.100060.

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