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Microbiota-gut-brain axis dysregulation in Alzheimer's disease and its modulation through probiotic supplementation

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Background: The microbiota-gut-brain axis (MGBA) has been implicated in the pathophysiology of Alzheimer's Disease (AD). Probiotics reduced the progression of AD in different mouse models, possibly through MGBA modulation, but human data are still limited.

Objective: Here, we evaluated whether differences in the gut microbiome (GM), pro-inflammatory markers and other MGBA mediators were associated with probable AD (pAD). We also assessed the impact of a 12-week probiotic treatment on MGBA.

Methods: Forty-five pAD patients and 47 healthy subjects (HC) were recruited at IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli of Brescia (Italy). An uncontrolled clinical investigation was performed to test the effects of 12-week probiotic supplementation in the pAD group. Fecal microbiota composition, intestinal and blood inflammatory markers, and microbiota-related metabolites were assessed before supplementation in all participants and after only in pAD.

Results: pAD patients showed intestinal inflammation, an altered GM profile, blood changes in the tryptophan metabolism, and reduced glutamate levels compared with HC (p-value< 0.049). Probiotic supplementation partially modulated these alterations, determining a reduction in several pro-inflammatory mediators, and an increase of GM-related protective factors, such as butyrate (p-value< 0.040) in pAD.

Conclusions: These findings confirmed the presence of MGBA alterations in AD and suggested a potential beneficial effect of probiotic supplementation through modulation of GM functionality rather than composition. Further research is required to confirm these results and their clinical relevance.

Key words: Alzheimer’s disease, probiotic supplementation, gut-microbiota-brain axis

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INTRODUCTION

Alzheimer's Disease (AD) is a widespread and highly disabling neurodegenerative disorder characterized by cognitive dysfunctions, memory loss, and emotional and functional impairments (Kamatham et al., 2024). At the molecular level, the pathology is characterized by the extracellular deposition of amyloid β fibrils and by the accumulation of intraneuronal neurofibrillary clusters of hyperphosphorylated tau protein (Roda et al., 2022). Alterations in the gut microbiota (GM) composition have been extensively reported in AD patients (Cattaneo et al., 2017; Vogt et al., 2017; Zhuang et al., 2018) and likely contribute to local and systemic inflammation, blood-tissue barrier permeability (Novoa et al., 2022; Sochocka et al., 2019) and to alterations of different gut metabolites that are known to impact brain functionality (Kesika et al., 2021; Sochocka et al., 2019).

Evidence that GM influences behavior and cognition and, in turn, that the brain modulates the GM profile, has contributed to the definition of the microbiota-gut-brain axis (MGBA), a bidirectional network where central and enteric nervous systems are linked through the same endocrine, immune, neural, and metabolic routes (Cryan et al., 2019). MGBA mediators that have been implicated in the pathophysiology of AD included cytokines and other mediators of immune responses, neurotransmitters, and bacterial metabolites such as short-chain fatty acids (SCFAs), tryptophan-derived active molecules, and bile acids (Cryan et al., 2019; Marizzoni et al., 2023; Meier-Stephenson et al., 2022; Mulak, 2021).

Prebiotics, probiotics, fecal microbiota transplantation, and diets have been proposed as possible adjuvant therapeutic interventions in AD (Varesi et al., 2022). For example, several preclinical data demonstrated the beneficial effect of such interventions on behavioral alterations and AD pathology through the modulation of the MGBA (Bonfili et al., 2017; Kaur et al., 2020; Prajapati et al., 2025). Moreover, recent meta-analyses using human data suggested that probiotic supplementation enhanced cognitive function in Mild Cognitive Impairment (MCI) and AD, likely through mechanisms involving the modulation of inflammation and oxidative stress as well as production of GM-related metabolites (Chui et al., 2024; Den et al., 2020; Xiao et al., 2025). Nevertheless, available evidence on the physiological effects of probiotics remains limited. To our best knowledge, the evaluation of a large panel of MGBA mediators in response to probiotics in probable AD (pAD) patients has not been described yet. A multidimensional approach assessing intestinal and circulating inflammation, microbiota composition, and metabolic alterations in response to GM-targeted treatment could help clarify the mechanisms through which the MGBA contributes to cognitive impairment and identify new therapeutic targets.

Therefore, in this study, we evaluated whether differences in the gut microbiome (GM), pro-inflammatory markers and other MGBA mediators were associated with probable AD (pAD), and whether these biological features could be modulated by a 12-week probiotic treatment.

Material and Methods

Study clinical cohort. Forty-seven cognitively healthy controls (HC) and forty-five probable AD (pAD) patients aged 55 to 89 years were recruited at IRCCS Istituto Fatebenefratelli San Giovanni di Dio in Brescia, between March 2018 and September 2019. Demographic and clinical characteristics were as expected for this population, with the exception of the older age of pAD patients compared with HC (p-value= 0.019, Table 1 and Supplementary Table 2).

Probable AD was defined based on the clinical evaluation according to established diagnostic criteria (Albert et al., 2011; McKhann et al., 2011). Both pAD and HC were community-dwelling people. To support the diagnosis of probable AD, both medical and family history were collected, alongside an assessment of previous episodes of cognitive decline. Exclusion criteria included antibiotic or anti-inflammatory treatment within 3 months prior to the collection of samples and the presence of a diagnosis of any psychiatric disorder. At baseline, participants underwent neuropsychological assessment through the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and the Clinical Dementia Rating (CDR) (Morris, 1993) scale, and completed a self-administered dietary questionnaire (Supplementary Method 1). Venous blood samples were collected using PAXgene™ Blood RNA Tubes (PreAnalytiX) and BD Vacutainers® (Becton Dickinson). Stool samples were collected from subjects at their own home in a sterile plastic cup, stored at −20°C for maximum 4 days, delivered to Istituto Fatebenefratelli San Giovanni di Dio, and kept at −20°C until sample processing (approximately for 280±177 days). The study was approved by the Ethics Committee of "Comitato Etico dell'IRCCS San Giovanni di Dio – Fatebenefratelli" (Brescia, Italy) under registration numbers 92/2017 and 95/2018. Written informed consent was obtained from all participants.

Explorative Intervention Study. In the context of this parent study, pAD patients were offered to participate in an explorative intervention study consisting of supplementation with a multispecies probiotic, Proxian®[1] (Errekappa Euroterapici). Proxian® was administered for 12 weeks in a stick formulation at a dosage of one stick per day. Each stick contained gastro-protected, microencapsulated probiotic strains *Bifidobacterium lactis BS05* (DSM 23032) and *Lactiplantibacillus plantarum LP01* (LMG P-21021), aimed at supporting gut microbiota balance. The formulation also included a non-viable tyndallized bacterial strain, *Lactobacillus buchneri LB26* (DSM 16341), naturally rich in Selenium and Sodium Selenite, with antioxidant properties. Additionally, Zinc was present to support cognitive function and immune response. pAD who agreed to participate in the explorative intervention study provided blood and stool samples at the end of the supplementation. MMSE scores were collected after probiotic supplementation but not in the same time window for all subjects, allowing the grouping into 3 categories: 1 month after the end of treatment, between 1 and 3 months, and after more than 3 months.

16S ribosomal RNA gene sequencing. Stool DNA was isolated, and the variable regions V3 and V4 of the bacterial ribosomal RNA 16S (rRNA 16S) gene were sequenced according to the 16S Illumina protocol. The raw 16S rRNA gene data were processed using QIIME2 (Bolyen et al., 2019) (Supplementary Method 2). Absolute abundances at the genus and phylum levels were normalized by the total number of reads assigned in each sample. Genera with incomplete taxonomy (i.e. 52 genera terminating at any level with '__'), assigned to the Eukaryota domain or found with relative abundance lower than 10^{-3} across all samples were removed.

Fecal Calprotectin. RIDASCREEN® Calprotectin (R-Biopharm Italia S.R.L.) immunoassay was performed on frozen stool samples, thawed immediately before the assay according to the manufacturer's protocol (Supplementary Method 3).

Blood biomarkers. Expression analyses of inflammatory genes (complete list in Supplementary Table 1) and the levels of four SCFAs (acetate, propionate, valerate and butyrate) were tested using Real-Time PCR and gas chromatography (GC), respectively (details in (Marizzoni et al., 2020) and Supplementary Methods 4 and 5). Compounds produced via tryptophan metabolism were quantified by liquid chromatography coupled to mass spectrometry (UPLC-MS) as previously described with some modification ((Mandal et al., 2024) and Supplementary Method 6).

Statistical analyses. Statistical analyses were performed using R (v.4.3.0) with Rstudio (2023.03.1+446), unless specified otherwise. Fecal calprotectin and blood variables were ln-transformed to assure normality, and the effect of clinical diagnosis at baseline was assessed using one-way ANOVA with age as covariate. Values that were above or below 2.5 standard deviations from the group mean were excluded from the analysis**.** Alpha and beta diversities were analysed using one-way ANOVA and permutational multivariate analysis of variance (PERMANOVA, R packages: vegan v2.6-4 (Oksanen et al., 2012)), respectively, with age and batch as covariates. Differential abundance of microbial genera between groups was assessed using linear mixed models implemented in MaAsLin2 (Microbiome Multivariable Associations with Linear Models, MaAsLin2 R package v 1.14.1) with age and batch as confounding variables.

For longitudinal analyses in pAD, linear mixed effect models with age and time as fixed effects and subject ID as random effect were applied for alpha diversity, fecal calprotectin, and blood variables after performing normality tests and after removing outliers. For alpha diversity, batch was also included as a covariate. For beta diversity, the effect of time was assessed using PERMANOVA with age and batch as confounding factors. Microbial genera associated with time or MGBA probiotic-related changes were evaluated using MaAsLin2.

To this end, delta of mediators found significantly changed after supplementation was dichotomized with 1 indicating increase (for glutamate, 5-hydroxy indole acetic acid, butyrate, and IL10) or reduction (for anthranilic acid, valerate, NLRP3, IL6, MIF, TNFalpha). MaAsLin2 was performed with time and dichotomized delta as fixed effects, and age, batch, and subject ID as random effects. Significance was set at $p < 0.05$ except for MaAsLin2, where an FDR lower than 0.25 (standard default used for this package) was applied. Changes in MMSE scores after supplementation were considered clinically significant as reported here (Muir et al., 2024): a change of 2 points for patients with MCI (CDR= 0.5) and mild AD (CDR= 1), a change of 3 points for patients with moderate to severe AD (CDR= 2 or 3). All figures were generated using Prism 10 (v 10.4.1) unless specified otherwise.

Results

Probable AD patients reported intestinal inflammation and alterations in the gut microbiota profile.

Our first aim was to evaluate the possible presence of an inflammatory status in the gut of patients in association with a specific gut microbiota composition profile. We measured the level of calprotectin, a well-known marker of gut inflammation, in fecal samples and we found that baseline levels were higher in pAD patients compared to HC (p-value< 0.001, Figure 1A). Then, we tested whether the presence of a peculiar GM profile was associated to pAD and we found differences in within-group Bray-Curtis and Jaccard distances that revealed lower inter-individual variation in pAD than in HC (PERMANOVA, p-value= 0.011 for both, Figure 1B), indicating that the individuals within the pAD group were more similar among them than those in the HC group. For alpha diversity, no differences were detected for Pielou's evenness and Shannon diversity (p-value> 0.352, Supplementary Figure 1A), indexes of richness and diversity of fecal microbiota, respectively. Although not statistically significant, pAD patients reported an increase in the phylum abundance of Bacteroidetes and Proteobacteria and a decrease in Firmicutes, Verrucomicrobia, and Actinobacteria compared to HC (Supplementary Figure 1B). At the genus level, pAD patients reported lower abundance of *Fusicatenibacter, Clostridium sensu stricto 1, Intestinibacter,* and higher abundance of *[Eubacterium] coprostanoligenes group, Rhodospirillales_uncultured, Desulfovibrio, CAG-352, Faecalitalea, and Negativibacillus* compared to HC (MaAsLin2, q-value<0.222, Figure 1C-D). These changes were irrespective of both stool storage duration and questionnaire-derived protein uptake, which was higher in pAD than HC (p= 0.031, Supplementary Table 3).

Probable AD patients had altered gut-related metabolites and inflammation in the blood

Having observed local inflammation and GM alterations in the gut of pAD subjects, we examined whether these were associated with changes in circulating MGBA mediators.

We found that pAD patients were characterized by significantly lower levels of glutamate and of bioactive compounds produced via tryptophan metabolism, namely tryptophan, xanthurenic acid, serotonin, and its metabolite 5-hydroxyindoleacetic acid (p-value< 0.029, Figure 2A-B). Conversely, anthranilic acid was increased in the pAD group compared to HC (p-value= 0.001, Figure 2B). A trend of decrease for kynurenine (p-value= 0.064) and 3-indole propionic acid (p-value= 0.083), as well as of increase for 3-hydroxykynurenine (p-value= 0.074) and nicotinamide (p-value= 0.062), was also observed in pAD than HC (Supplementary Figure 1C). While no differences were detected for SCFAs (Supplementary Figure 1D), increased expression of the pro-inflammatory cytokine IL-8 (p-value= 0.048, Figure 2C) and a trend for TNFalpha (p-value= 0.097, Supplementary Figure 2F) was found in pAD than HC.

Probiotic treatment modulated microbiota-related metabolites and inflammation

Next, we tested whether probiotic supplementation for 12 weeks could counteract the specific biological alterations of MGBA in pAD patients. When we tested whether MGBA could be modulated by a 12-week probiotic treatment, an increasing trend was observed in the Pielou's evenness index (p-value= 0.070, Supplementary Figure 2A), while no significant changes were observed in the Shannon diversity index as well as in the microbiota structure as indicated by the Bray-Curtis and Jaccard distances (p-value> 0.05). However, probiotic-driven modulations were identified at the genus level, where we reported a decreased abundance of *Ruminococcaceae UCG-004* and *Faecalitalea* (q-value< 0.141, Figure 3A). Notably, *Faecalitalea* was found to be increased in pAD compared to HC at baseline (q-value= 0.222, Figure 1).

Concerning microbiota-related metabolites, probiotic supplementation decreased valerate and anthranilic acid (p-value< 0.040) and increased butyrate, 5-hydroxyindoleacetic acid, and glutamate (p-value< 0.036, Figure 3B-D). A trend of increase was reported for tryptophan, kynurenine and kynurenic acid (p-value< 0.100, Supplementary Figure 2F). Furthermore, an effect on peripheral inflammation was detected in the blood, where probiotic reduced the levels of several pro-inflammatory cytokines, namely IL6, TNFalpha, NLRP3, MIF (p-value< 0.009, Figure 3E), and increased those of the anti-inflammatory cytokine IL10 (p-value< 0.001, Figure 3F). The mean fecal calprotectin levels were reduced after probiotic consumption although did not reach a significant difference (p-value= 0.413, Supplementary Figure 2B).

Next, we assessed whether microbiota genera could be associated with the significantly increased or decreased levels of MGBA mediators and we found 11 genera as significantly associated with such blood changes (q-value< 0.020, Figure 3G): 5 genera correlated with decreased levels of mediators involved in inflammatory processes (*CAG-56* with NLRP3; *Ruminococcus-2* with IL6, valerate and anthranilic acid; one uncultured bacteria belonging to the Puniceicoccaceae family with MIF and anthranilic acid; *Marvinbryantia* with anthranilic acid), 5 with increased levels of the known beneficial metabolite butyrate (*Ruminococcus-2*, *Ruminococcaceae-UGC-002*, *-003*, *-005* and *Dorea*), *Eisenbergiella* with increased levels of 5-hydorxy indole acetic acid and, an uncultured bacteria belonging to the Barnesiellaceae family, with increased levels of glutamate. The strongest correlations were observed for those genera associated with increased butyrate (5.19< FC< 5.26, 0.024< q-value< 0.069), decreased IL6 (FC= -9.01, q-value= 0.069) and anthranilic acid (-7.67< FC< -6.18, 0.053< q-value< 0.085).

Finally, probiotic supplementation exerted a positive influence on cognition for 4 pAD patients 1 month after the end of treatment, and for 2 pAD patients after more than 3 months (Figure 4). No patients showed worsening at follow-ups apart from 2 subjects with CDR of 1 at baseline, who reported, respectively, a reduction of 2 and 5 MMSE points over 3 months after the end of supplementation.

Discussion

The main findings of the present study confirmed that pAD is characterized by the presence of intestinal and peripheral inflammation, of microbiota composition alterations and of a dysregulation of GM-related metabolites, including those related to tryptophan metabolism and the neurotransmitter glutamate. We also showed that 12-week probiotic supplementation in pAD patients can reduce peripheral inflammation and increase the circulating levels of beneficial microbiota-related metabolites, and that these blood changes are associated with the fecal microbiome composition.

When we looked at microbial differences in patients versus control, we found, among others, a reduction of *Clostridium sensu stricto 1* and *Intestinibacter,* and an increase in *Eubactrium coprostanoligenes, Rhodospirillales,* and *Desulfovibrio,* which are in line with previous findings (Cammann et al., 2023; Grabrucker et al., 2023; Haran et al., 2019; Ling et al., 2020; Marizzoni et al., 2023; Quattrini et al., 2025; Vogt et al., 2017). Furthermore, pAD patients showed a decrease of *Fusicatenibacter,* generally considered beneficial for intestinal health and it has been found associated with the anti-inflammatory effects in patients with rheumatoid arthritis, a chronic autoimmune disease (Yu et al., 2021). Moreover, pAD patients reported increased abundance of *CAG-352* and *Faecalitalea* than controls supporting the suggested role for *CAG-352* in increasing vulnerability to stress (Chen et al., 2022) and for *Faecalitalea* in modulating insulin response in diabetes (Ma et al., 2020), a well-known risk factor for AD.

The intestinal and systemic inflammation that we found in pAD patients confirm previous findings from our group and others (Grabrucker et al., 2023; Leblhuber et al., 2015) and were in line with the knowledge that peripheral inflammation is potentially harmful in the context of chronic activation (Pagoni et al., 2022; Walker et al., 2021). In addition to inflammatory molecules, microbial metabolites are other MGBA mediators known to affect brain function and behaviour (Cryan et al., 2019). Recent evidence has pointed to the involvement of tryptophan metabolism in AD pathogenesis with emphasis on the role of some tryptophan metabolites in modulating immune responses and inhibiting the amyloid oligomerization and aggregation (Meier-Stephenson et al., 2022). Indeed, tryptophan is metabolized into various bioactive compounds through three main pathways: the kynurenine, the serotonin, and the indole pathways. Here we reported, in line with literature data, a blood reduction of tryptophan (Whiley et al., 2021) and the dysregulation of all its downstream pathways in pAD, as discussed below. Regarding the kynurenine pathway, other studies identified lower plasma levels of xanthurenic acid in AD patients (Giil et al., 2017; Whiley et al., 2021) as well as higher anthranilic acid in preclinical AD (Oxenkrug, 2024). Moreover, the blood alterations of these two metabolites, along with glutamic acid, have been associated with a greater risk of dementia in dementia-free participants followed over 10 years (Chouraki et al., 2017). Glutamic acid is the neutral form of glutamate and, as reported here, was found decreased in plasma (here and in (Chang et al., 2021)) and brains of AD patients (Antuono et al., 2001; Hattori et al., 2002; Rupsingh et al., 2011). Regarding the serotonin pathway, we found a decrease in serotonin and its metabolite 5-hydroxyindoleacetic acid in the blood. A reduction of both these compounds has been extensively reported in the blood, cerebrospinal fluid, and brains of AD patients (Morimoto et al., 2017; Sekiya et al., 2024). Lower serotonin levels have been associated with higher hazards of functional decline and incident mild behavioral impairment in subjects with no dementia (Sim et al., 2025). The indole pathway is primarily a result of microbial metabolism in the gut. One of its main metabolites, the 3-indolepropionic acid, is known to have anti-amyloid and neuroprotective properties *in vitro* and animal models of AD (Bendheim et al., 2002; Chyan et al., 1999; Hwang et al., 2009). Consistently, we found a trend of decreased 3-indolepropionic acid in pAD patients compared with controls. Nevertheless, another study reported higher levels of this metabolite in MCI progressing to AD compared to MCI that remained stable during an observation period of approximately 5 years (Huang et al., 2021), suggesting that additional research is needed to fully elucidate the role of indoles and the other tryptophan-derived metabolites in the different stages of AD. Besides tryptophan metabolism and glutamate, we also evaluated SCFAs, but we failed to identify any effect of diagnosis on their concentration, likely due to the lower number of healthy subjects (N=10) included in the baseline analysis. Taken together, the evidence above supports the hypothesis that some of these MGBA molecules may influence the onset and progression of AD and are central to the symbiotic relationship between intestinal microbes and the brain. Nevertheless, longitudinal studies are necessary to confirm which ones.

Another important aim in this study was to evaluate whether probiotic supplementation for 12 weeks could counteract alterations observed in pAD at baseline as compared to controls and could also modulate molecules with important roles in the MGBA. Our exploratory intervention study showed that, despite no apparent modulation of the GM composition, probiotic supplementation induced changes in metabolites produced (i.e. SCFAs) or whose production is influenced (i.e. indoles) by the microbiota. This is in line with the current notion that probiotics may exert their effects through transient colonization of the gut microbiota (Zmora et al., 2018), modulating the activity and interactions of other resident microbes (Grazul et al., 2016), as well as directly influencing immune and inflammatory mechanisms as the communication between T cells and dendritic cells (Cristofori et al., 2021). Here, probiotics reduced the levels of several molecules involved in immune activation processes, as pro-inflammatory cytokines (i.e. IL6, MIF, NLRP3, TNFalpha), valerate and anthranilic acid (Shaw et al., 2023), as well as increased potential protective factors, as anti-inflammatory cytokines (i.e. IL10), butyrate, and indoles (Chakraborty et al., 2024; Chyan et al., 1999). All these beneficial effects have been extensively reported in AD preclinical models (Kaur et al., 2020; Li et al., 2024; Song et al., 2022; Sun et al., 2020; Sun et al., 2021; Zhu et al., 2023), but to our best knowledge, this is the first study that has measured all of these players in patients with pAD to provide an overall picture.

Most of the available AD clinical studies evaluated the effect of probiotics on cognition (Akhgarjand et al., 2022; Fei et al., 2023; Xiao et al., 2020) or brain atrophy (Asaoka et al., 2022); few studies assessed peripheral inflammation reporting a general anti-inflammatory effect, measured as reduced IL1beta (Hsu et al., 2023), C-reactive protein (Tamtaji et al., 2019), TNFalpha, IL8, and IL12p70 (Ton et al., 2020); one study evaluated tryptophan and kynurenine and, in line with the our results, identified an increase in kynurenine concentrations in AD patients after probiotic (Leblhuber et al., 2018). Our results also suggest, for the first time, a beneficial effect of probiotic supplementation in restoring serotonin and glutamate to control group levels, as well as in increasing butyrate and decreasing valerate. Human data on blood levels of serotonin, glutamate, and SCFAs after probiotic intake are lacking, but previous research has suggested that diet and GM composition might impact the bioavailability of all of them (Gruenbaum et al., 2024; Socała et al., 2021). The rebalance of serotonin could improve some symptoms associated with the disease, such as agitation and irritability, possibly a consequence of its decrease in AD patients as compared with controls (Siddique et al., 2009). While alterations in glutamate signaling are significant aspects of AD (Zott & Konnerth, 2023), whether the glutamate coming from diet and microbial sources might contribute to disease pathophysiology remains to be explored. We previously found that high plasma butyrate and low plasma valerate were associated with endothelial integrity and low brain amyloidosis in older persons with cognitive performance from normal to dementia (Marizzoni et al., 2020). Together with the fact that butyrate is critical for epithelial integrity (Mathewson et al., 2016) and that valerate has been linked with several neurodegeneration-related biomarkers (pTau-181, neurofilament light chain and glial fibrillary acidic protein) (Marizzoni et al., 2025), these findings suggest that probiotic-induced modulation could lead to beneficial effects on some key peripheral processes in AD. It should be noted that this and current studies exhibit considerable methodological heterogeneity and variability (i.e., probiotic used, disease stage, biomarkers evaluated), along with small sample sizes. Therefore, more well-designed and controlled studies are required to elucidate the complex connection between microbiome-modulating interventions and AD.

This study has several limitations. The uncontrolled design of the intervention study precludes definitive conclusions about causality on the effects of the probiotic. However, these findings reveal a clear modulation of specific analytes following probiotic administration, highlighting their potential as outcome measures in future trials. We did not systematically collect cognitive evaluations after the supplementation, which prevents us from drawing any inference about the relationship between peripheral probiotic modulation and cognitive improvement. Despite this, results on the MMSE showed that 4 of 12 patients with moderate AD evaluated in the month after the end of supplementation showed clinical improvement, and none worsened within three months. Moreover, we have not used biomarkers to confirm the presence of amyloid and tau pathology, nor evaluated the probiotic effect on them. Furthermore, the cellular composition of blood samples was not evaluated, and it cannot be excluded that the differences observed in gene expression were partly due to changes in the abundance of immune cells. Lastly, since 16S rRNA gene sequencing did not allow for the functional characterization of bacteria, we included the quantification of microbiota-related metabolites to obtain functional information on the microbiota.

In conclusion, our findings confirmed the dysregulation of the microbiota-gut-brain axis in AD through intestinal and systemic inflammation and imbalances of microbiota-related metabolites involved in the behavioral and cognitive alterations of the disease. Moreover, we demonstrated the potential beneficial effect of probiotic supplementation in counteracting these changes, mainly by reducing pro-inflammatory mediators and increasing protective factors. Finally, we suggest that the gut microbiota is a valid target in AD for inducing profound peripheral changes, although further research is needed to confirm these findings and their clinical relevance.

Availability of data and materials

The 16S rRNA sequencing raw data supporting the conclusions of this manuscript are publicly available in the European Nucleotide Archive, reference number PRJEB89799. The microbiota-gut-brain axis marker dataset analysed during the current study is available from the corresponding author on reasonable request.

Author contributions

MM: Conceptualization, Formal analysis, Methodology, Writing - original draft. EM: Methodology, Investigation, Data curation, Project administration, Writing - original draft. SA: Methodology, Investigation. MR, PM, LC, DL, SS, DS, FP, VB: Investigation. DVM, MS: Resources. GBF: Validation. AC: Conceptualization, Resources, Validation, Supervision. All: Writing - review & editing.

Disclosure statement

The authors report no disclosures.

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Table 1. Sociodemographic and clinical data of study participants. Figure denotes mean ± SD and number (%). Abbreviations: APOEe4, ε4 allele of the apolipoprotein E; HC, healthy controls; pAD, patients with probable Alzheimer's disease.

[1] Missing data for 2 HC and 1 pAD; [2] Missing data for 3 HC and 4 pAD; [3] Missing data for 2 HC and 2 pAD.

	HC	**pAD**	**p-value**
N	47	45	
Age (years)	70.8 ± 9.6	75.7 ± 7.8	0.019
Females (N, %)	26 (55)	31 (69)	0.180
APOEe4 carrier status (N, %)	13 (28)	23 (51)	0.032
Education[1] (years)	10.6 ± 5.0	7.1 ± 3.1	<0.001
Body mass index[2] [Kg/cm^2]	26.1 ± 4.2	24.6 ± 3.8	0.140
Mini-Mental State Examination score[3]	29.1 ± 1.1	18.2 ± 5.6	<0.001

Figure 1. Baseline fecal calprotectin (A) and GM (B-D) comparison between pAD and HC. Data are presented as boxplots with black horizontal lines indicating medians and circles indicating the subject's values. Abbreviations: HC, healthy controls; pAD, patients with probable Alzheimer's disease.

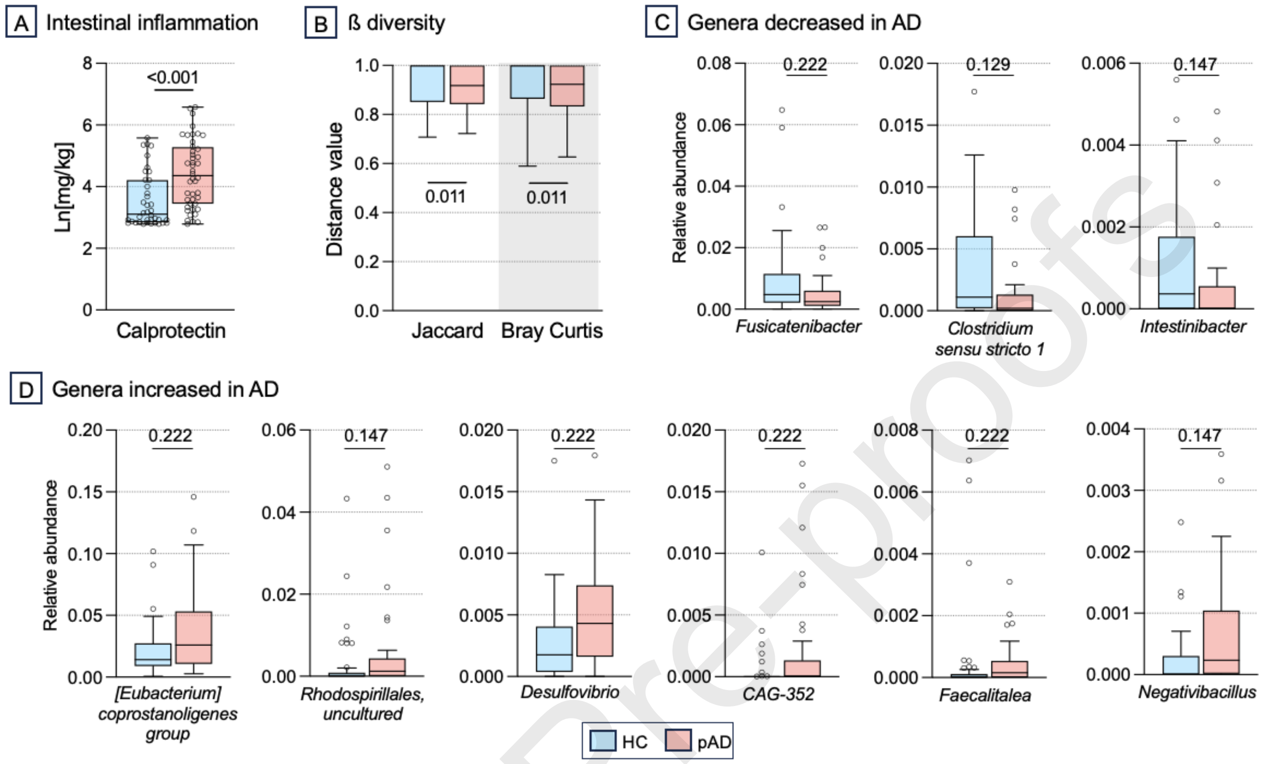


Figure 2. MGBA mediator comparison between probable AD and HC, namely plasma glutamate (A), bioactive compounds produced via tryptophan metabolism (B), and the pro-inflammatory cytokine IL8 (C). Data are presented as boxplots with black horizontal lines indicating medians and circles indicating the subject's values. Abbreviations: HC, healthy controls; pAD, patients with probable Alzheimer's disease.

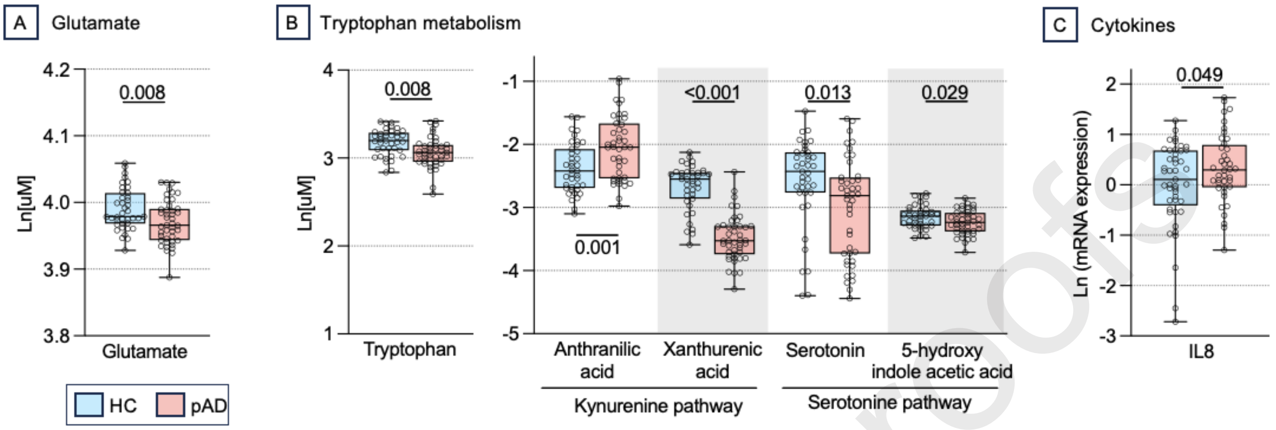


Figure 3. Probiotic treatment modulated microbiota, its metabolites, and inflammation in probable AD. Data are presented as spaghetti plots with the mean per time-point for microbial genera (A), glutamate (B), bioactive compounds produced via tryptophan metabolism (C), short-chain fatty acids (D), and blood gene expression levels of pro- (E) and anti-inflammatory (F) cytokines. Heatmap summarizing all the significant associations between microbial, cytokine, and SCFAs changes as calculated using linear mixed models (G). Color key: -log(q-value) * sign (coefficient). Cells that denote significant associations are colored (red or blue) and overlaid with an asterisk (* sign indicating a q-value < 0.2). The correlation heatmap was visualized using the corrplot package (v0.95).

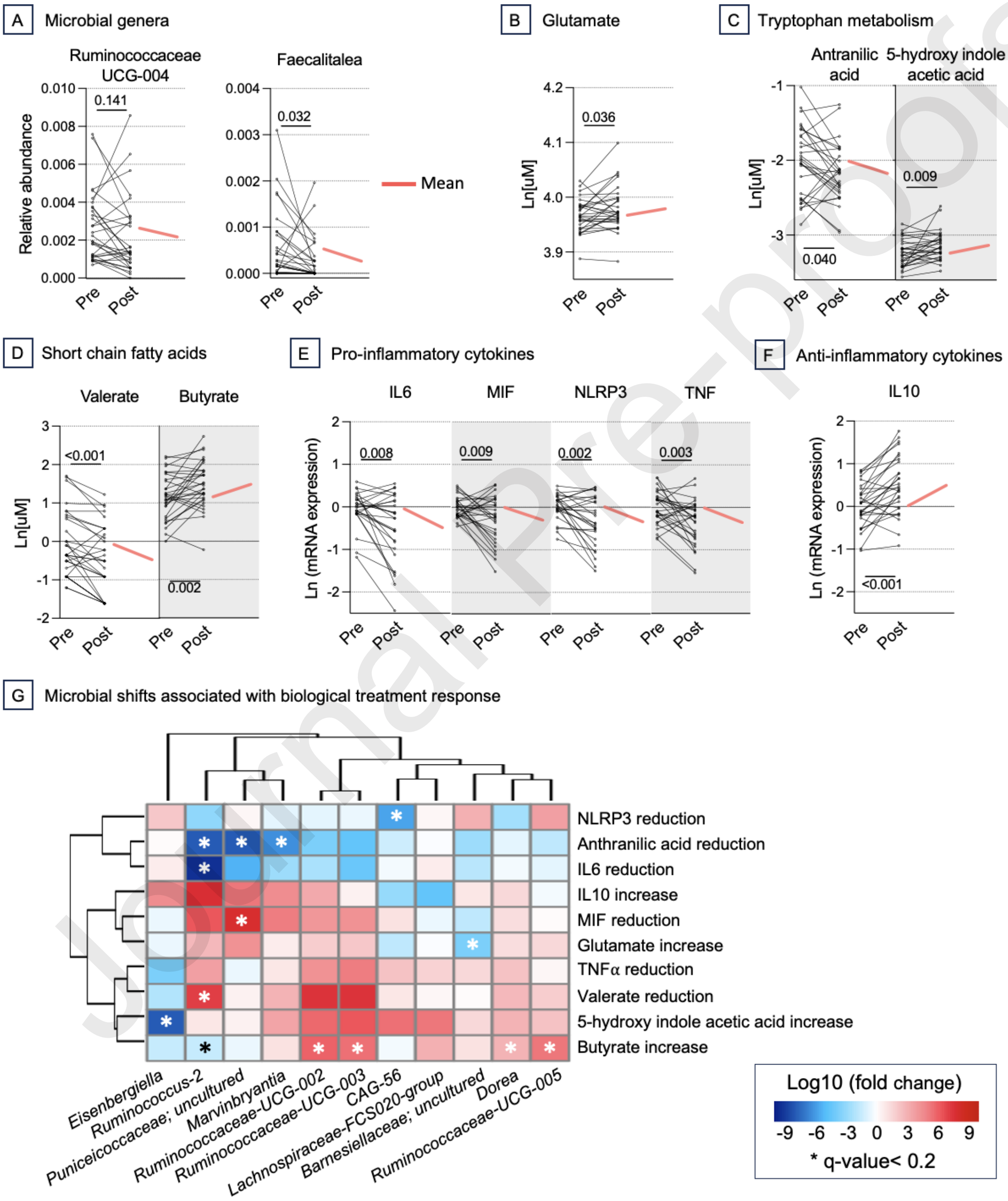
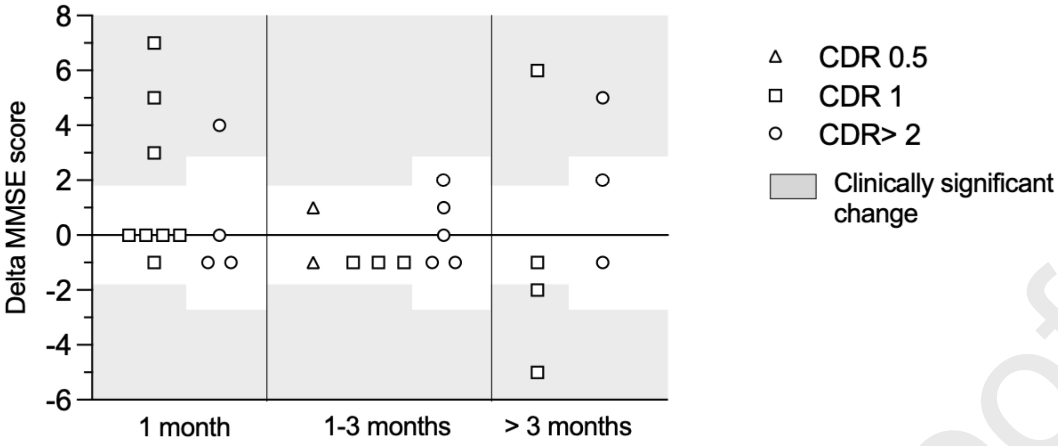


Figure 4. Pre- and post-treatment Mini-Mental State Examination differences in probable AD patients. Each symbol corresponds to a patient. The gray background corresponds to a clinically significant change as reported in (Muir et al., 2024): for patients with CDR score equal to 0.5 and 1, a change of 2 points was considered meaningful, and for patients with

CDR score of 2 or 3, the change had to be at least 3 points. Abbreviations: CDR, Clinical Dementia Rating scale scores.



Highlights

- Alzheimer patients show peripheral inflammation and metabolic dysregulation
- Probiotics reduce inflammation and increase beneficial metabolites in the periphery
- Probiotic-induced peripheral changes are associated with microbiome profile