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Theoretical Article

Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota

Binyin Li[1], Yixi He[1], Jianfang Ma, Pei Huang, Juanjuan Du, Li Cao, Yan Wang, Qin Xiao, Huidong Tang**, Shengdi Chen*

Department of Neurology & Collaborative Innovation Center for Brain Science, Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, P.R. China

Abstract

Objective: Gut microbiota changes before the onset of Alzheimer's disease (AD) and the alterations could be detected in the stage of mild cognitive impairment (MCI). The findings might offer diagnostic biomarkers before the onset of dementia.

Background: AD is the most common cause of dementia, and MCI is the predementia state. Recent studies suggest the alterations in the gut microbial communities associated with AD, whereas the microbiota in MCI before the onset of dementia has not been discovered and characterized in humans.

New/Updated Hypothesis: We hypothesize that the dysbiosis happens in the MCI stage. Patients with AD and MCI have decreased microbial diversity, and changes in gut microbiota could be detected for early detection of AD. In our preliminary study, we identified differences between AD and normal controls in 11 genera from the feces and 11 genera from the blood. No difference in genera between AD and MCI was detected. Using the diagnostic model from fecal samples with all different genera input, 93% (28 in 30) of patients with MCI could be identified correctly.

Major Challenges for the Hypothesis: The diagnosis of MCI and AD in the study was based on symptoms and neuroimaging, and AD biomarkers should be included for precise diagnosis in further validating studies. Besides, as the microbiota changes longitudinally, their relationship with the progress of dementia needs to be studied in the prospective studies.

Linkage to Other Major Theories: *Escherichia* was observed increased at genus level in both fecal and blood samples from AD and MCI. For AD biomarker, postmortem brain tissue from patients with AD showed lipopolysaccharides and gram-negative *Escherichia coli* fragments colocalize with amyloid plaque. In this way, the amyloid pathogenesis for AD would be triggered during MCI by gut microbiota shifting. Besides, systemic inflammatory reactions caused by compounds secreted by bacteria may impair the blood-brain barrier and promote neuroinflammation and/or neurodegeneration. Furthermore, abnormal metabolites caused by microbial gene functions have an impact on neurodegeneration.

Keywords: Mild cognitive impairment; Alzheimer's disease; Fecal microbiota; 16S rRNA gene; Amyloid

1. Objective

This article is a proposal for a new hypothesis on the gut-brain axis in Alzheimer's disease (AD) based on our novel findings. The present draft intends to (1) promote new thinking about the role of gut microbiota in the pathogenesis of AD and (2) solicit the presence of AD-like gut microbiota change in mild cognitive impairment (MCI), to attract more attention to its value in early diagnosis. This effort also aims

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[1]These authors contributed equally to this work.

*Corresponding author. Tel.: 86-21-6445-4473; Fax: 86-21-6445-4473.

**Corresponding author. Tel.: 86-21-6445-4473; Fax: 86-21-6445-4473.

E-mail addresses: chensd@rjh.com.cn (S.C.), funground@126.com (H.T.)

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to assess the role of microbiota in the pathogenesis of neurodegeneration and thus to identify accessible biomarkers.

2. Background

2.1. Historical evolution

AD causes irreversible dementia [1], and MCI due to AD is a clinical state before the onset of dementia. Various pathological changes, such as accumulation of amyloid-β protein (Aβ) and tau, happen several years before the symptoms [2]. The detection of these biomarkers requires positron emission tomography or lumbar puncture, and few people could accept these radioactive or invasive tests when no or only mild symptom was observed. Gut microbiota, which could be analyzed from fecal samples, was suggested to be involved in neurodegenerative disease through the gut-brain axis [3,4]. The gut-brain axis has been considered as a possible cause of AD, via a bidirectional communication system such as neural, immune, endocrine, and metabolic pathways [5]. In the transgenic AD mouse model, AD pathology shifted gut microbiota composition toward an inflammation-related bacterial profile, suggesting that these changes could contribute to disease progression and severity [6]. The diet also impacted the AD progression. Germ-free mice demonstrated deficits in nonspatial and working memory tasks, as well as reduced expression of the brain-derived neurotrophic factor in the hippocampus [7]. In humans, recent studies have investigated gut bacterial taxa and found altered abundance in patients with AD [8,9].

2.2. Rationale

The clarification of the gut-brain axis in AD pathogenesis would provide accessible biomarkers from feces. However, the current clinical studies for AD gut microbiota did not uncover whether microbiota has already changed before the onset of dementia. In addition, the role of blood circulation as a potential communication system in the gut-brain axis is also unclear. The findings in AD gut microbiota indicate there is a need to explore whether similar changes occur in the MCI stage. It is very likely that the involvement of the gut-brain axis could be detected several years before dementia, which provides the rationale for early diagnosis. Such a transformation in thinking will also need new conceptions about biochemical mechanisms that happen in the gut-brain axis.

3. New or updated hypothesis

3.1. Early experimental or observational data

The regulatory role of gut microbiota in AD pathogenesis has been greatly studied. Patients with AD had decreased diversity indexes in gut and blood microbiota compared with normal controls [8,10,11].

Postmortem brain tissue from patients with AD showed lipopolysaccharides (LPS) and gram-negative *Escherichia coli (E. coli)* fragments colocalize with amyloid plaque [12]. Regarding the mechanisms, a variety of microbiome generates functional amyloids, which help form microbial communities such as biofilms [13–15]. The *E. coli* extracellular amyloids composed of the major structural subunit CsgA could thus facilitate surface attachment and adhesion, biofilm development, and protection against host defenses [16,17]. Besides, bacterial amyloid systems also include some gram-negative species of *Streptomyces, Bacillus, Pseudomonas, Staphylococcus,* and others [16–18]. More importantly, microbial and cerebral AD amyloids have similar higher-order structure, pathogen-associated molecular pattern composition and physic-chemical characteristics [14]. They could be recognized by the same toll-like receptor (TLR) 2/TLR1 receptor system as $A\beta_{42}$ and produce proinflammatory cytokines [13,14]. Regarding the pathway for pathogenetic factors to the brain, Braniste et al. [19] found the relationship between the lack of normal gut flora in germ-free mice and increased blood-brain barrier (BBB) permeability in adult and embryonic animals. The disruption or absence of the microbiota in mice impaired the function of the BBB and induced abnormal central nerve system (CNS) function, including cortical myelination, hippocampal neurogenesis, cognitive function, and memory formation [20]. In this way, systemic inflammatory reactions caused by compounds secreted by bacteria may impair the BBB and promote neurodegeneration [21–23]. The proinflammatory cytokines released from gram-negative bacteria is capable of mediating amyloid aggregation and inflammatory signaling [24,25].

Besides the direct amyloid accumulation and its triggered neuroinflammation in AD, the systemic inflammation caused by microbial dysbiosis has been shown to exacerbate this effect. *Lactobacillus*, *Bacteroides*, and *Prevotella* were negatively correlated, while *Bifidobacterium* was positively correlated with an approximate increase in intestinal permeability [26]. The increase in intestinal permeability coincided with higher plasma LPS, serum IL-1, and TNF-α. Clinical studies suggested that individuals with microbial dysbiosis caused by intestinal diseases would develop AD with a great risk [27,28].

As diet has a positive effect on changing microbiota composition [29], the changes in AD diet (such as appetite, lower frequent shopping) may interfere with the results. In a recent prospective clinical study, patients with AD were given mixtures of probiotics containing both *Lactobacillus* and *Bifidobacterium* genera. However, probiotic supplementation has an insignificant effect on either cognitive or biochemical indications in patients with severe AD [30].

In the following preliminary study, we recruit participants with similar diet habit and then compare the microbiota in patients with AD and MCI and normal controls to minimize the confounding factor.

Table 1
Clinical and demographic data of patients with AD and MCI and cognitively normal controls

Characteristics	AD patients (n = 30)	MCI patients (n = 30)	Normal controls (n = 30)	*P* value
Age, year (SD)	66.3 (5.1)	65.4 (7.6)	63.9 (5.1)	.298
Male, n (%)	15 (50.0)	12 (40.0)	13 (43.3)	.643
Education, year (SD)	11.0 (3.5)	12.4 (2.8)	11.6 (3.3)	.081
BMI, mean (SD)	23.0 (3.5)	23.2 (2.9)	24.03 (2.9)	.231
MMSE scores, mean(SD)	18.1 (8.2)*	27.2 (1.1)	29.1 (1.2)	<.01
Constipation (%)	3 (10)	1 (3.3)	3 (10)	.402
Diabetes (%)	2 (6.7)	3 (10)	2 (6.7)	.881

NOTE. Age, education, BMI, and MMSE scores are expressed as means (SD). Sex and constipation are expressed as a proportion; *P* value, analysis of variance, or χ^2 test.

Abbreviations: AD, Alzheimer's disease; BMI, body mass index; MMSE, Mini–Mental State Examination; SD, standard deviation.

*The MMSE of the AD group is significantly lower than other two groups measured by the post hoc test.

3.2. Preliminary results/findings

We hypothesize the insignificant difference between AD and MCI and analyze the microbiota communities in the feces and blood of patients with AD and MCI and normal controls by 16S rRNA sequencing (inclusion and exclusion criteria in the Supplementary Material). The diagnosis of AD and MCI was based on the criteria of the National Institute on Aging–Alzheimer's Association (NIA-AA) workgroups [9]. We first compared the microbiota between three groups. Moreover, we explored the relationship between the severity of AD and the microbiota and validated the prediction model for MCI identification, which was established based on data from patients with AD and normal controls.

The demographic and clinical characteristics of patients with AD and MCI and normal controls were of no significant differences in age, gender, education, BMI, or constipation (Table 1). As expected, patients with AD had a lower Mini–Mental State Examination score than patients with MCI and normal controls (18.1 ± 8.2 vs 27.2 ± 1.1 and 29.1 ± 1.2, respectively, $P < .01$, Table 1), whereas patients with MCI and normal controls did not differ with respect to Mini–Mental State Examination. Patients with AD had disease duration of 2.8 ± 2.6 years (from the onset of dementia) and took acetylcholinesterase inhibitors (donepezil) and/or memantine, whereas patients with MCI were not given these or other medications. No selective serotonin reuptake inhibitors were used for all patients.

In the fecal samples, sequencing of the V3-V4 region of the 16S rRNA gene generated a total of 3.99 million sequence reads (mean ± standard deviation: 44,431 ± 6513 reads/participant). The final operational taxonomic unit (OTU) data set for AD, MCI, and control groups consisted of 710 OTUs classified to 143 genera, 50 families, 28 orders, 19 classes, and 11 phyla. In the blood samples, the OTU data set consisted of 760 OTUs, classified to 275 genera, 117 families, 75 orders, 44 classes, and 23 phyla.

In the fecal microbiota, only one of the mean community diversity indexes in the AD group, phylogenetic diversity (PD) whole tree, was significantly lower than that of the normal controls (Fig. 1A–D). Post hoc Nemenyi's test showed no significant difference between AD and MCI ($P = .941$). Other indexes of α-diversity (including chao1, observed species, shannon based on OTU levels) were similar between three groups. Although in the blood microbiota, more indexes (PD whole tree, chao1, observed species, shannon) of the AD group were significantly lower than those of the controls (Fig. 1F–I). Similarly, no significant difference of α-diversity in blood microbiota was found between AD and MCI.

For β-diversity, significant differences were also observed based on the weighted (qualitative, ANOSIM R = 0.345, $P = .001$) and the unweighted (quantitative, ANOSIM R = 0.22, $P = .001$) UniFrac between the fecal samples from three groups, as well as those from the blood samples (ANOSIM R = 0.346, $P = .001$ for weighted, and R = 0.727, $P = .001$ for unweighted UniFrac). The differences between AD and MCI were not significant in both fecal and blood samples.

Based on the linear discriminant analysis effect size method, we compared the genera between the patients with AD and cognitively normal controls from fecal and blood samples, respectively. Considering differences in the taxa at the genus level with a logarithmic linear discriminant analysis score >2.0 and *P* value < .01, we found that the abundance of 7 genera in the fecal microbiota (*Lactobacillus, Akkermansia, Dorea, Bifidobacterium, Streptococcus, Acinetobacter,* and *Blautia*) was higher in the AD group, whereas the abundance of 11 genera *(Parabacteroides, Alistipes, Bacteroides, Alloprevotella, Haemophilus, Paraprevotella, Succinivibrio, Sutterella, Prevotella, Barnesiella,* and *Butyricimonas)* was lower (Fig. 2A). In the blood samples, the AD group had 10 genera (*Acidovorax, Escherichia, Pelagibacterium, Stenotrophomonas, Propionibacterium, Glutamicibacter, Pseudomonas, Sulfuritalea, Vibrionimonas,* and *Staphylococcus*) of higher abundance, whereas had 12 genera (*Serratia, Ochrobactrum, Halomonas, Brevundimonas, Nesterenkonia, Methylobacterium, Aliihoeflea, Leucobacter, Achromobacter, Acinetobacter, Enterobacter,* and *Pannonibacter*) of lower abundance (Fig. 2B). After adjusted for possible confounding factors (age, gender, BMI, and

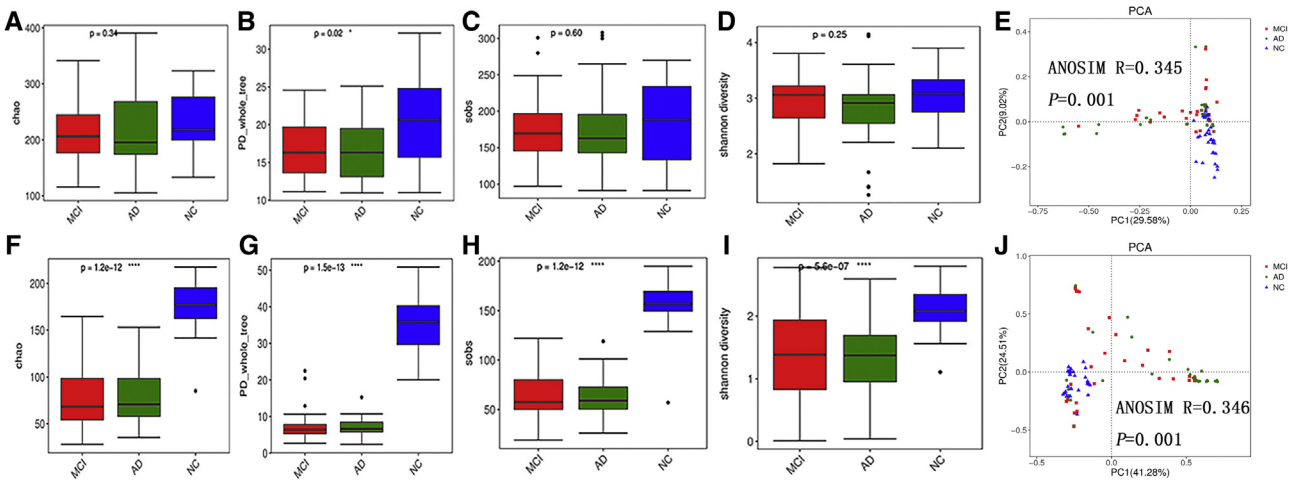

Fig. 1. The α-diversity and β-diversity indices of the fecal and blood microbiota in patients with AD and MCI and normal controls. (A-D) Box plots depict differences in the fecal α-diversity indices according to the Chao 1 index, PD whole tree index, observed species index, Shannon index based on the OUT counts. Each box plot represents the median, interquartile range, minimum, and maximum values. (F-I) Box plots depict α-diversity in blood microbiota in the three groups. E & J: Weighted ANOSIMs and PCA based on the distance matrix of UniFrac dissimilarity of the fecal (E) and blood (J) microbiota communities in the AD, MCI, and NC groups. ANOSIM R values showed the community variation between the compared groups, and significant *P* values are indicated. The axes represent the two dimensions explaining the greatest proportion of variance in the communities. Each symbol represents a sample, and each line connects a pair of samples. Abbreviations: ANOSIM, analyses of similarities; AD (green), Alzheimer's disease; MCI (red), mild cognitive impairment; NC (blue), normal controls; PCA, principal coordinates analysis.

constipation) by general linear models, 11 genera in the feces (increased in AD: *Dorea*, *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Blautia*, and *Escherichia*; decreased in AD: *Alistipes, Bacteroides, Parabacteroides, Sutterella,* and *Parprevotella*) and 11 genera in the blood were of different abundance between two groups (increased in AD: *Propionibacterium, Pseudomonas, Glutamicibacter, Escherichia,* and *Acidovora;* decreased in AD: *Acinetobacter, Aliihoeflea,*

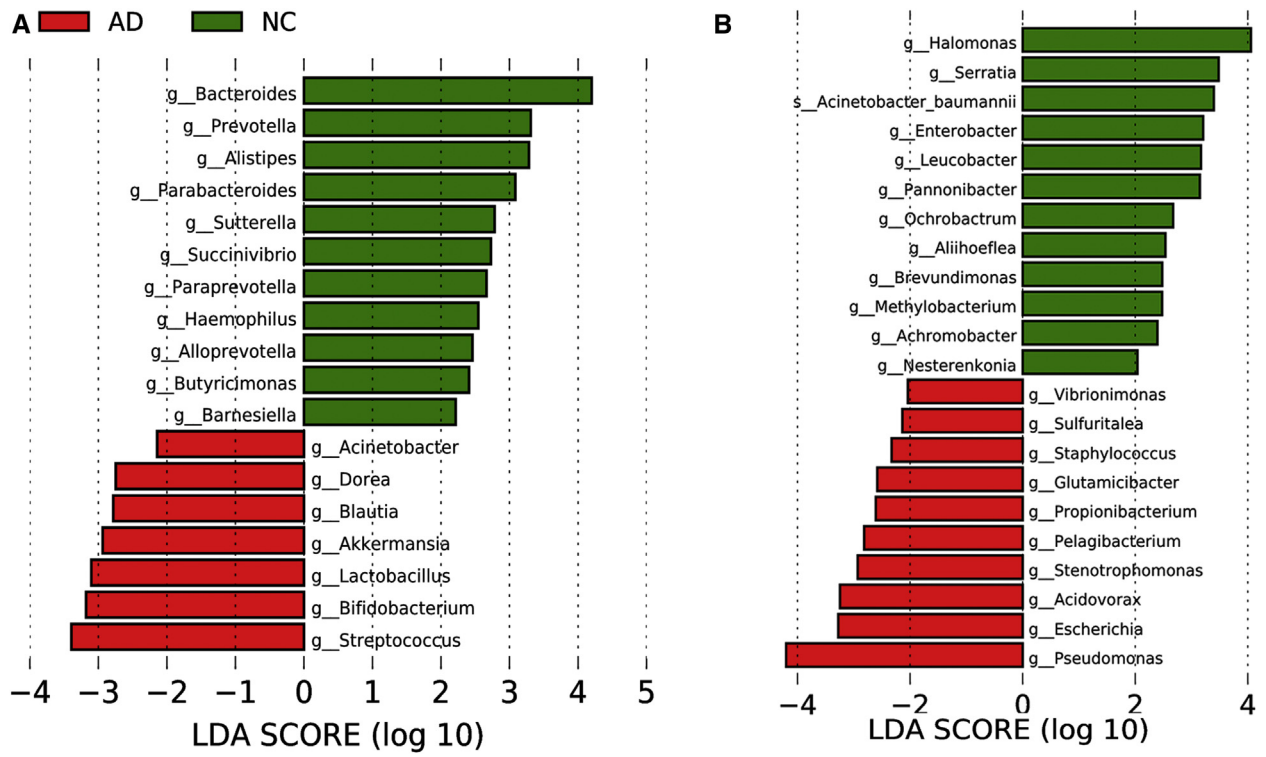

Fig. 2. Taxonomic differences of fecal microbiota in patients with AD and MCI and normal controls. (A) Linear discriminant analysis (LDA) effect size (LEfSe) analysis revealed significant bacterial differences in fecal microbiota between the patients with AD (negative score) and normal controls (positive score). (B) Significant bacterial differences in blood microbiota. The LDA scores (log10) > 2 and $P < .05$ are listed. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment.

Table 2
Difference between patients with AD and cognitively normal controls at the genus level after confounding factors adjusted for

AD versus NC	Genus	B value (95% CI)	*P*
Feces	*Alistipes*	−5.76 (−8.46 to −3.05)	<.001
	Bacteroides	−1.31 (−1.72 to −0.90)	<.001
	Parabacteroides	−7.65 (−11.77 to −3.53)	<.001
	Sutterella	−12.84 (−21.26 to −4.42)	.002
	Paraprevotella	−7.80 (−15.05 to 0.55)	.003
	Escherichia	1.27 (0.64 to 1.90)	<.001
	Blautia	12.82 (5.11 to 20.53)	.001
	Bifidobacterium	4.61 (1.67 to 7.74)	.002
	Streptococcus	3.28 (1.17 to 5.39)	.002
	Lactobacillus	3.41 (0.49 to 6.34)	.022
	Dorea	9.75 (0.48 to 19.01)	.039
Blood	*Acinetobacter*	−1.65 (−3.09 to 0.21)	.025
	Aliihoeflea	−37.10 (−53.48 to 20.72)	<.001
	Halomonas	−1.54 (−2.06 to −1.02)	<.001
	Leucobacter	−18.89 (−23.56 to −14.22)	<.001
	Pannonibacter	−21.30 (−26.27 to −16.33)	<.001
	Ochrobactrum	−8.07 (−14.20 to −1.94)	.001
	Acidovorax	8.63 (4.98 to 12.29)	<.001
	Escherichia	4.77 (2.28 to 7.25)	<.001
	Glutamicibacter	24.25 (12.62 to 35.88)	<.001
	Pseudomonas	0.94 (0.60 to 1.28)	<.001
	Propionibacterium	18.96 (7.56 to 30.38)	.001

NOTE. Result of the GLMs for significant genera (sequence counts) based on the group factors (AD and cognitively normal controls) and possible confounding factors (age, gender, BMI, and constipation) of 60 individuals. The positive b value indicated the taxa were associated with AD patients, and negative number indicated the taxa were associated with the normal controls.

Abbreviations: AD, Alzheimer's disease; CI, confidence interval; GLM, general linear model; NC, normal control.

Halomonas, Leucobacter, Pannonibacter, and *Ochrobactrum*) ($P < .05$, Table 2). Among 6 increased genera in AD feces, four of them were in the phylum Firmicutes, whereas genera in the phylum Bacteroidetes dominated the decreased genera. Quantitative polymerase chain reaction confirmed the increased *Escherichia* and *Lactobacillus* and *decreased Bacteroides* in AD and MCI feces (Fig. 3). We also found the significantly negative relationship between amyloid burden and relative abundance of *Lactobacillus* (Supplementary Table 5).

In the feces, the abundance of *Akkermansia* positively correlated with medial temporal atrophy (MTA). The higher abundance of *Fusicatenibacter*, *Blautia*, and *Dorea* (in the family Lachnospiraceae) was associated with lower MMSE and that of *Faecalibacterium, Butyricicoccus*, and *Hungatella* (in the family Clostridiaceae) was related with higher MMSE. Regarding disease duration, higher abundance of *Megamonas* was positively correlated with longer duration (Fig. 4A).

In the blood, the abundance of *Glutamicibacter* was positively correlated with the cognitive ability, whereas negatively correlated with MTA. A positive association was observed between the abundance of *Aeromonas, Rubrobacter*, and MTA. Higher abundance of *Streptococcus, Chryseobacterium, Corynebacterium, Brevibacterium,* and *Rubrobacter* were significantly associated with longer disease duration (Fig. 4B).

We constructed the random forest model based on the fecal and blood difference between patients with AD and normal controls, using the significantly different abundance taxa at the genus level from general linear model results as the input. In fecal models, the mean area under the receiver operating characteristic curve was 0.781 for each genus, and it was 0.864 in blood data models. Using the cutoff values from random forest models with all different genera fecal input, 28 of 30 patients with MCI could be identified correctly, with sensitivity 93% and classification error 6.3%.

According to OUT and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) [31], we predicted the functional categories based on a comparison of the Kyoto Encyclopedia of Genes and Genomes (KEGG) ortholog (KO) between AD patients and normal controls. In the level 2 KEGG pathways, the microbial gene functions related to metabolisms of amino acid, carbohydrate, energy, glycan, lipid, cofactors and vitamins, nucleotide, terpenoids, and polyketides were significantly lower in the AD group.

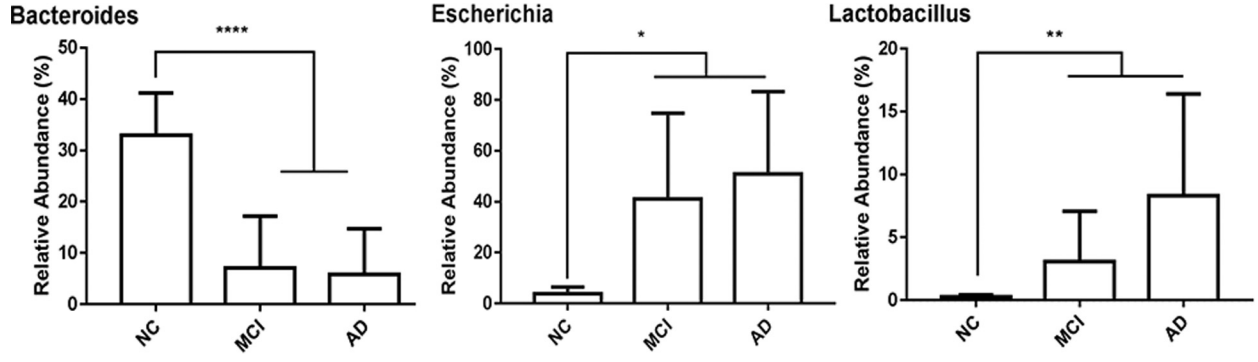

Fig. 3. The relative abundance of *Bacteroides, Escherichia,* and *Lactobacillus* measured by qPCR (ratio between the genera and 16S rRNA gene) were compared between three groups by the Kruskal-Wallis test. Post hoc comparison was performed by Dunn's test. No significant difference was found between AD and MCI. ****$P < .0001$; **$P < .01$; *$P < .05$. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; NC, normal controls; qPCR, quantitative polymerase chain reaction.

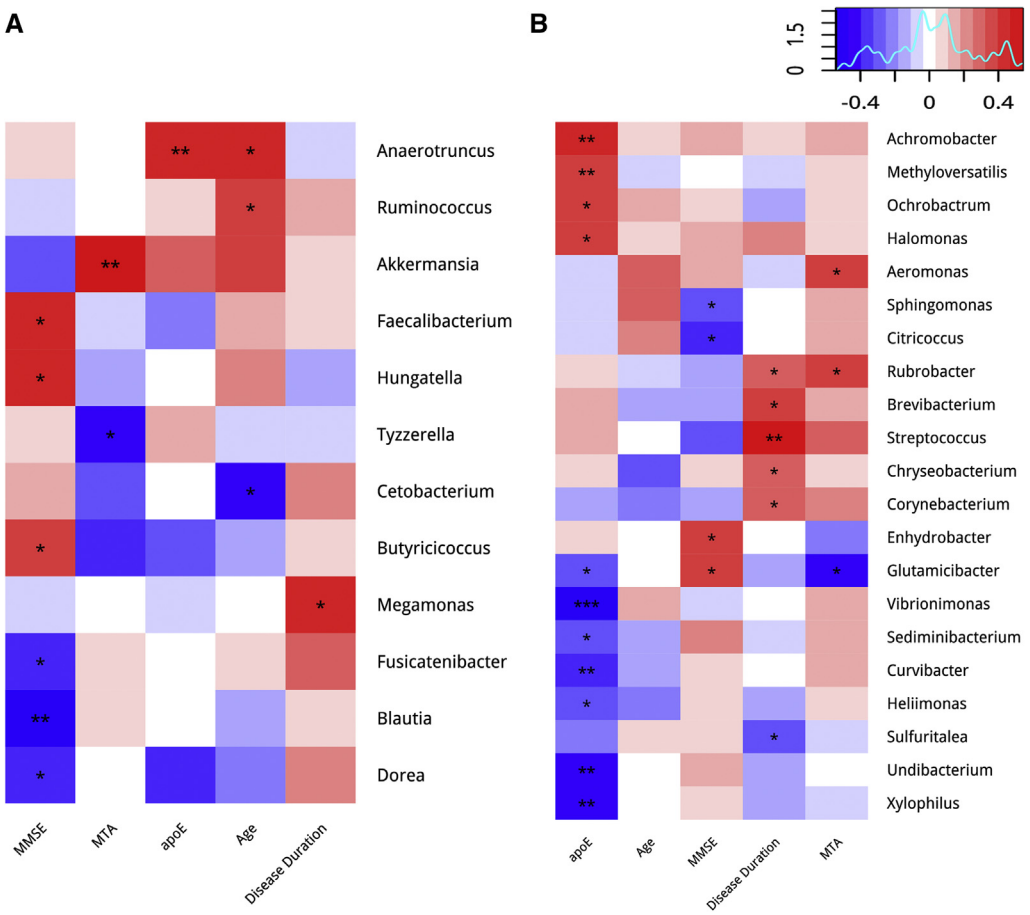

Fig. 4. Heatmaps showing correlations between microbiota genera and AD clinical characteristics. Heatmap based on the abundance (sequence counts) of the microbiota genera (prevalence > 10% in patients with AD) showed the correlations between the fecal (A)/blood (B) microbiota and AD clinical characteristics. *APOE* was the genotype and expressed as categorical data. We set value 2 for ε4/ε4 carriers, 1 for single ε4 carriers, and 0 for non-ε4 carriers. Abbreviations: AD, Alzheimer's disease; MMSE, Mini-Mental State Examination, MTA, medial temporal atrophy.

In conclusion, we observed evidence for gut microbiota dysbiosis in the patients with AD and MCI (Fig. 5). MCI presented similar fecal and blood microbiota as AD, and it would enhance the understanding of pathogenesis in predementia state and help identify risky population.

3.3. Further experiments or validation studies

In the aforementioned preliminary study, the similarity between MCI and AD suggested that human gut microbiota could change before the onset of dementia. Microbiota may contribute to the accumulation of Aβ in the CNS, affecting neuroinflammation by modulation of several neurochemical and neurometabolic pathways.

First, follow-up of MCI or mild AD cohort and tracking the changes of microbiota could help to validate biomarkers from changed microbiota for AD or MCI. It is equally important to evaluate circulating inflammatory factors and pathogenetic metabolites from microbiota. Second, a detailed analysis of taxa function and methods from ecology should be introduced to uncover net effect of the entire microbiota, as well as bidirectional communication involving neurotransmitters and hormones. Familywise error should be minimized in the case-control studies.

In clinical trials about AD and gut microbiota changes, no direct evidence suggests the effect of current medications (acetylcholinesterase inhibitor or N-methyl-D-aspartate antagonist) for AD on the brain-gut axis [32]. If we want to know whether the anti-AD medications change gut microbiota, the comparison between AD with or without these medications should be performed in further clinical trials.

Moreover, based on results obtained from the aforementioned studies, prospective clinical trials for probiotic supplementation in diet as an intervention for patients with mild AD or MCI should be performed for further validation. For a comprehensive evaluation of effect, multi-model assessments are required in trials, including cognitive tests, neuroimage, and AD biomarkers (amyloid and/or tau). Larger research studies from different populations with different diet habits are still needed to confirm the results.

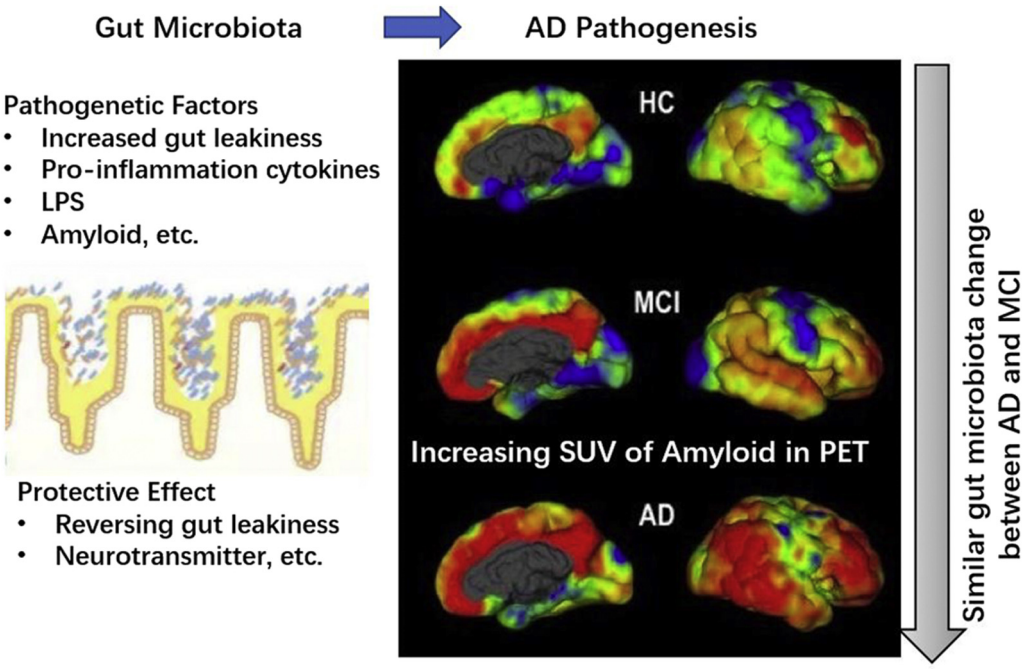

Fig. 5. The figure illustrated the hypothesis that both pathogenetic and protective effect from gut microbiota changes could happen in the early stage of AD. The markers found among the changes help early diagnosis of MCI due to AD. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment.

4. Major challenges for the hypothesis

Based on our current findings, we hypothesize that MCI patients have similar alterations of gut microbiota composition to AD, and the microbiota is different from that in cognitively normal controls. Though MCI has a much slighter decline in cognitive abilities than AD, the alterations may happen several years before the onset of dementia. The normal controls did not show AD or MCI-like pattern microbiota, which helps differentiate the normal aging and AD. One of the challenges comes from the lack of biomarker tests. Though MCI patients had memory complaints and mild temporal atrophy, it was worthwhile to find whether they had positive pathological biomarkers, such as Aβ or tau.

Another challenge comes from the validation of the relationship between microbiota changes and AD pathogenesis and severity. From fecal samples of patients with AD and MCI, we observed an increase in the genus *Escherichia*, *Blautia*, *Bifidobacterium*, *Streptococcus*, *Lactobacillus* and *Dorea*. Among them, the abundance of *Blautia* and *Dorea* found in the gut were associated with lower MMSE scores. We hypothesize the *Blautia* and *Dorea* in phylum Firmicutes as risk factors in the AD pathogenesis. Occupancy of *Blautia* and *Dorea* at the genus level increased in the secrete membrane vesicles of AD mouse model, as well as Firmicutes at the phylum level in the APP/PS1 mice and *Drosophila* model [10,33]. In this way, systemic inflammatory reactions caused by compounds secreted by bacteria may impair the BBB and promote neurodegeneration [21–23]. *Escherichia* may be another risk factor, as it increased at genus level in both fecal and blood samples from AD and MCI. Owing to the evidence of LPS and *E. coli* fragments colocalizing with amyloid plaque, we suggest that the risky amyloid pathogenesis for AD could be triggered during MCI by gut microbiota shifting. Further studies would investigate the relationship between all-domain cognitive performance, amyloid plaque size, and bacterial products, which would help validate this speculation.

Bacteroides might be one of the protective factors in microbiota. Patients with AD and MCI exhibited a decreased abundance of the genus *Bacteroides* [9,24], which could be explained by its ability to protect intestinal barrier and reversing gut leakiness [34].

The relationship between genera and causes of dementia is also a challenge. The different genera found between AD and normal controls did not correlate with MTA or apolipoprotein E (*APOE*) genotype. Besides, other causes of dementia, such as frontotemporal degeneration and progressive supranuclear palsy, have similar pathological changes such as paired helical filaments of tau with 3 or 4 repeats in the microtubule-binding domain. Quantitative tau by positron emission tomography has been shown to correlate with cognitive performance in AD even more robustly than amyloid [35]. To precisely make the early diagnosis, it would be helpful that the microbiota analysis should also be performed between AD and other related causes of dementia. In addition, certain strains of *Lactobacillus* and *Bifidobacterium* secrete gamma-aminobutyric acid (GABA) that may mediate the positive GABAnergic function for cognition in AD [36]. The increase of *Lactobacillus* in AD feces [37] might be a kind of gut-brain self-protective effect.

5. Linkage to other major theories

The critical finding in our hypothesis was the similarity between AD and MCI. The changes of gut microbiota in MCI suggest the dysbiosis long before the onset of dementia, characterized by overrepresentation of gram-negative bacteria [38].

The mechanism for the adverse effect of dysbiosis on the brain could be related to amyloid, neural, and even systemic inflammation. Regarding β amyloid, several bacterial species, including *Bacillus subtilis, E. coli, Mycobacterium tuberculosis, Salmonella enterica, Salmonella typhimurium, and Staphylococcus aureus*, were capable of producing amyloid fibers [23,39,40]. Amyloid fibers were capable of crossing the intestinal barrier, as well as the BBB [41,42], and amyloid protein could be deposited in the CNS and promote AD pathogenesis [43]. In humans, Cattaneo et al. found a higher abundance of *Escherichia/Shigella* in amyloid-positive patients, and these gut microbiota differences were correlated with the systemic inflammatory profile [24].

Furthermore, because the expression of functional *TREM2* is downregulated in some late-onset AD cases, the diminished protective effect from *TREM2* may exacerbate proinflammatory responses induced by toxins such as LPS from *E. coli* [44], leading to Aβ clearance deficiency [45]. On the contrary, gut microbiota is also able to reduce Aβ load in AD, and certain bacteria may help reduce AD pathology. Microbial grape-seed polyphenol extracts have been shown to interfere with the oligomerization of Aβ [46].

Regarding the neuroinflammation, we have mentioned the effect of gut microbiota on intestinal permeability and they could trigger systemic proinflammatory cytokines. The systemic inflammation could act synergistically to accelerate AD pathology [47]. On the other hand, the antibiotic-induced normalization in gut microbiota could reverse the inflammatory process and might alter the risk for developing AD [26].

Besides the risk of amyloid pathogenesis and proinflammatory cytokines from gut bacteria, we found that the microbial gene functions related to metabolism and biosynthesis of fatty acids were overactivated in the AD and MCI gut microbiota. It was suggested that acetate as one fatty acid could cross the BBB, decrease its permeability, and also affect microglia [48]. Otherwise, antibiotic-induced microbial dysbiosis had been shown to reduce colonic levels of acetate, butyrate, propionate, adenine, and uracil. More detailed studies should give insight into these metabolites' effect in AD pathogenesis.

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Data availability: The high-throughput sequence data have been deposited in the National Center for Biotechnology Information (NCBI) BioProject database with project number PRJNA489760. All other data are available on request from the authors.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2019.07.002.

RESEARCH IN CONTEXT

1. Systematic review: Fecal and blood samples were collected from Alzheimer's disease, mild cognitive impairment, and healthy controls. We compared the microbiota communities in the feces and blood by 16S rRNA gene amplicon, sequencing, and validation, as well as evaluating correlations between microbiota and clinical characteristics and amyloid deposition quantitatively.
2. Interpretation: We suggest that the alterations in gut microbiota may happen several years before the onset of dementia, even in the mild cognitive impairment stage. The healthy controls did not show Alzheimer-like pattern microbiota, which helps differentiate the normal aging from early stage of AD.
3. Future directions: It is worthwhile to find the relationship between specific pathological biomarkers and microbiota changes. It would help uncover the underlying mechanism of the impact from the microbiota to AD pathogenesis.

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