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Short-Chain Fatty Acids and Lipopolysaccharide as Mediators Between Gut Dysbiosis and Amyloid Pathology in Alzheimer's Disease

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Abstract.

Background: Metagenomic data support an association between certain bacterial strains and Alzheimer's disease (AD), but their functional dynamics remain elusive.

Objective: To investigate the association between amyloid pathology, bacterial products such as lipopolysaccharide (LPS) and short chain fatty acids (SCFAs: acetate, valerate, butyrate), inflammatory mediators, and markers of endothelial dysfunction in AD.

Methods: Eighty-nine older persons with cognitive performance from normal to dementia underwent florbetapir amyloid PET and blood collection. Brain amyloidosis was measured with standardized uptake value ratio versus cerebellum. Blood levels of LPS was measured by ELISA, SCFAs by mass spectrometry, cytokines by using real-time PCR, and biomarkers of endothelial dysfunction by flow cytometry. We investigated the association between the variables listed above with Spearman's rank test.

Results: Amyloid SUVR uptake was positively associated with blood LPS (rho ≥ 0.32, $p \leq 0.006$), acetate and valerate (rho ≥ 0.45, $p < 0.001$), pro-inflammatory cytokines (rho ≥ 0.25, $p \leq 0.012$), and biomarkers of endothelial dysfunction (rho ≥ 0.25, $p \leq 0.042$). In contrast, it was negatively correlated with butyrate (rho ≤ -0.42, $p \leq 0.020$) and the anti-inflammatory cytokine IL10 (rho ≤ -0.26, $p \leq 0.009$). Endothelial dysfunction was positively associated with pro-inflammatory cytokines, acetate and valerate (rho ≥ 0.25, $p \leq 0.045$) and negatively with butyrate and IL10 levels (rho ≤ -0.25, $p \leq 0.038$).

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Conclusion: We report a novel association between gut microbiota-related products and systemic inflammation with brain amyloidosis via endothelial dysfunction, suggesting that SCFAs and LPS represent candidate pathophysiologic links between the gut microbiota and AD pathology.

Keywords: Brain amyloidosis, inflammation, lipopolysaccharide, microbiota, short chain fatty acids

INTRODUCTION

Alzheimer's disease (AD) pathology is the most common cause of neurodegenerative dementia, featuring extra neuronal accumulation of amyloid-β (Aβ), and intraneuronal deposition of hyperphosphorylated tau. However, despite great progress in understanding the dynamics of the molecular pathology, treatments targeted at AD pathology and its modifiers have not yet resulted in a disease modifying therapy.

There is clear evidence that AD is characterized by the presence of a pro-inflammatory status both in the periphery, in term of higher blood levels of pro-inflammatory cytokines, and in the brain, in term of activated microglia, the resident innate immune cells of the central nervous system (CNS). Moreover, Aβ has recently been recognized as an antimicrobial peptide, as part of the innate immune system [1] and, while monomeric Aβ shows little antimicrobial activity, its capability of aggregation allows to form antimicrobial pore-forming structures [2].

Based on this, researchers have recently proposed a potential role for the gut microbiome in the initiation and exacerbation of AD pathology and several preclinical and clinical studies indeed support this hypothesis. For example, in 2016, for the first time, Minter and colleagues reported that antibiotic-induced perturbations in the gut microbiota (GMB) diversity influence neuroinflammation and amyloidosis in a murine model of AD [3]. Subsequently other studies by using the sequencing of bacterial 16S ribosomal RNA from fecal samples of APP transgenic mice revealed significant differences in the GMB composition compared to that of control wild type mice [4, 5]. Interestingly, in the paper of Harach and collaborators, the authors found also that cerebral Aβ was significantly reduced in germ-free APP transgenic mice and that their recolonization with microbiome coming from conventionally raised APP transgenic mice increased cerebral Aβ pathology, and this increase was less effective when wild type mice microbiota was used. Preclinical data have been also supported by clinical data, where the presence of alterations in the composition of the gut microbiome have reported in AD patients as compared to control individuals [6, 7]. Our research group in 2016 demonstrated an increased abundance of proinflammatory *Escherichia/Shigella* and decreased abundance of anti-inflammatory *Eubacterium rectale* in patients with cognitive impairment and brain amyloidosis and also an association of their abundances with peripheral inflammation [8]. A subsequent study that used a 16S sequencing approach revealed that the GMB profile in AD patients is characterized by a reduced microbial diversity, decreased abundance of *Firmicutes* and *Bifidobacterium* and an increased abundance of *Bacteroidetes*; interestingly, the relative bacterial abundance correlated with the increase of cerebrospinal fluid markers of AD pathology [7].

There are several pathways through which the gut bacteria may influence brain functioning and promote neurodegeneration in the context of AD pathology. One of these biological processes is represented by inflammation: microbes are known to influence the development and regulation [9] of the immune system and, therefore, may alter the interaction between the immune and the nervous systems. The gram-negative membrane protein lipopolysaccharide (LPS), which has pro-inflammatory properties, was found in amyloid plaques and around vessels in AD brain [10]. Moreover, LPS levels have been found significantly higher in plasma samples of AD patients as compared to controls [11], and a positive association was found between LPS levels and degree of blood monocyte/macrophage activation. Importantly, a pro-inflammatory status has been identified as among the most important determinants of endothelium dysfunction [12]. Indeed, changes in the gut microbiome composition could lead to alterations in the functionality of endothelial cells, which are key cellular players of inflammatory reactions and strictly interact with the soluble factors involved in the regulation of each of the different steps of the inflammatory reaction; such endothelial dysfunction could then promote or enhance the

spread of the inflammatory status from the gut to the blood and to the brain. The GMB is also known to produce several metabolites through fermentation on non-digestible carbohydrates with neuroprotective properties [13] that directly or indirectly affect brain functions. Among these, short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, are known to beneficially modulate the peripheral and central nervous systems and have been already suggested to play a central role in AD [14]. In particular, it has been hypothesized that SCFAs may attenuate AD by serving as substrates for energy metabolism [15] and providing an alternative energy source to counteract the brain hypo-metabolism that contributes to neuronal dysfunction in AD [16]. Recent evidence also suggests that SCFAs may help to modulate maturation and function of microglia in the brain [17]. More recently, it was also shown that butyric acid may provide therapeutic benefits for AD through epigenetic mechanisms of action by inhibiting histone deacetylase and normalizing aberrant histone acetylation [18]. This evidence suggests that the production of SCFAs in the gut may represent another biological mechanism through which the gut microbiome may influence AD.

In this study we aimed at evaluating whether inflammatory mediators and bacterial products are potential pathogenic links between the GMB and amyloid pathology in AD via endothelial dysfunction. To this purpose, we tested the associations of brain amyloidosis with blood levels of LPS, SCFAs, cytokines, and several markers associated with endothelial dysfunction in a cohort of elderly subjects with cognitive performance ranging from normal to dementia.

MATERIALS AND METHODS

Study participants

Participants were Italian community-dwelling persons of 50 to 85 years of age recruited from a large Italian study on amyloid imaging in patients with cognitive complaints, the Incremental Diagnostic Value of [18F]-Florbetapir Amyloid Imaging [INDIA-FBP] study [19]. Inclusion criteria were cognitive abnormality, age between 50 and 85 years, and availability of an informant (spouse, adult child, or another knowledgeable informant). Cognitively impairment was defined as follows: 1) presence of cognitive complaints reported by patients or proxy or by the doctor; 2) presence of no intracranial metabolic or psychiatric causes of cognitive impairments; 3) presence of abnormal scores in $\geq$2 cognitive tests; and 4) history of progression of cognitive symptoms. The clinical evaluation included the collection of medical cognitive, functional, and drug history, and physical and neurological examination. The cognitive assessment of each participant included the Mini-Mental State Examination (MMSE) and the Alzheimer's Disease Assessment Scale, cognitive portion (ADAS-Cog). In the context of this parent study, 150 patients and controls who were not under antibiotic and anti-inflammatory treatment over the past 3 months or had been diagnosed with major depression or other psychiatric disorders were proposed to contribute samples of stools and blood. Accepting patients signed an ad-hoc informed consent. Venous blood was sampled from an antecubital vein between 08:00–10:00 h am and collected in EDTA tubes (BD Vacutainer Systems, Franklin Lakes, NJ).

[^{18}F]-Florbetapir amyloid PET

As previously reported [8], amyloid PET was performed at the Nuclear Medicine Service of Spedali Civili and Fondazione Poliambulanza in Brescia with GE Discovery 690 and Siemens Biograph 40 m PET–computed tomography scanners, respectively. PET was a 10-min (two 5-min frames) 3-dimensional acquisition, 50 min after the injection of an intravenous bolus of 370 MBq (10 mCi) of ^{18}F-Florbetapir [20]. Attenuation correction was calculated based on the co-acquired computed tomography. PET images were reconstructed onto a 128 $\times$ 128 matrix with slice thickness of 3–3.3 mm, using a 2- to 3-mm Gaussian post-reconstruction filter. Standardized uptake value ratios (SUVRs) were calculated in the frontal, parietal, temporal, anterior cingulate, posterior cingulate, and precuneus regions of interest as well as global measure.

Inflammatory mediators

Isolation of total RNA was performed using the PAXgene blood miRNA kit according to the manufacturer's protocol (PreAnalytiX, Hombrechtikon, CHE). RNA quantity and quality were assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrophotometer (NanoDrop Technologies, Delaware, USA) and by using the Agilent BioAnalyzer (Agilent Technologies) and samples were stored at –80°C until processing. Gene expression analyses on candidate genes was

performed in RNA samples from blood circulating cells (obtained by using PaxGene tubes) using semi-quantitative real-time PCR and included a panel of inflammatory mediators known to be involved in AD. In particular, the gene expression measurement was performed for the pro-inflammatory mediators IL1β, NLRP3, IL6, CXCL2, CXCL10, TNFα, and IL18 as well as for the anti-inflammatory mediators IL10 and IL4. Each sample was assayed in duplicate, and each target gene was normalized to the expression of three reference genes, glyceraldehyde 3-phosphate dehydrogenase, beta-actin, and beta-2-microglobulin, using Taqman Assays on a 384 wells Real Time PCR System (Biorad). The expression levels of each target gene were normalized to the geometric mean of all three reference genes, and the Pfaffl method was used to determine relative target gene expression of each gene in patients as compared with controls. The primer sequence for each of the genes is available on request to the authors.

GMB-related products

LPS was measured in plasma by ELISA (Pierce LAL Chromogenic Endotoxin Quantitation Kit, Thermo Fisher Scientifics) and SCFAs levels (i.e., acetate, propionate, valerate, and butyrate) using gas chromatography (GC) according to the following procedure. Briefly, after thawing at room temperature (RT), 400 microliters of plasma were deproteinized by the addition of 50 microliters of 16% (W/V) metaphosforic acid and mixed by vortexing for 30 s. After, an incubation at 60°C for 30 min was performed to completely precipitate the protein fraction. Successively, all samples were centrifuged at 8000 rpm for 30 min, the supernatant was collected and filtered at 0.2 micron. The SCFA determination was performed using the GC DANI 1000 (Dani instruments S.p.A. Monza Italy) equipped with split-splitless injector and HP-FFAP megabore water compatible capillary column (length 30 mt with 0.53 mm internal diameter; Agilent Technologies INC, CA, USA). Iso-valerianic acid was used as internal standard. The GC conditions were Injector 200°C and F.I.D. (detector) 260°C. Column temperature setting was: initial temperature 85°C, hold time 0.5 min, PRGM1 column heating rate 7°C/min, PRGM1 final column temperature 135°C, hold time 0.1 min, PRGM2 column heating rate 25°C/min, PRGM2 final column temperature 160°C, hold time 4.0 min. For the concentration of each acid, three standard solutions have been prepared: 1) low 80 ppm; 2) medium 120 ppm; 3) high 200 ppm) with 100 ppm of I.S. for each one. Response Factor calculation was determined as follow:

$$RF = \text{I.S. peak area} \times [\text{V.F.A.}] / \text{V.F.A.} \times [\text{IS}]$$

The concentration of each VFA was determined by applying the following formula:

$$[\text{VFA}] = 100 \times \text{VFA peak area} \times \text{VFA RF} / \text{I.S. peak area}$$

The results were expressed in micromoles/liter.

Endothelial dysfunction markers

The LEGENDplex™ Human Adhesion Molecule Panel multiplex assay (Cat.#740945, BioLegend) was used to measure the plasma level of the following cell adhesion molecules (CAMs): intracellular CAM 1, 2 and 3 (ICAM-1, ICAM-2, and ICAM-3, respectively), vascular CAM (VCAM-1), platelet endothelial CAM 1 (PECAM-1), activated leukocyte CAM 1 (ALCAM-1), epithelial CAM (EpCAM), neural CAM (NCAM), endothelial selectin (E-selectin), platelet selectin (P-selectin), leukocyte selectin (L-selectin), platelet endothelial CAM 1 (PSGL-1), and CD44. Briefly, the surface of each fluorescent bead set is conjugated with specific capture antibodies so that they can be considered as capture beads. The fluorescent capture beads are incubated in 1:50 diluted human plasma samples containing the target molecules for 2 hr at room temperature in a V-bottom 96 well plate in continuous shaking in the dark. Successively, after 2 wash steps, a biotinylated detection antibody mix (25 mL) is added to each well and incubated for 1 h at RT in continuous shaking. In this way, each detection antibody will bind to its specific analyte bound on the capture beads (capture bead-analyte-detection antibody sandwich). Then, a 25 mL volume of streptavidin-phycoerythrin (SA-PE) solution is subsequently added and incubated for 30 min at RT. The binding to the biotinylated detection antibodies will provide a fluorescent signal (FL2 channel) intensities in proportion to the amount of bound analytes. After a final wash step, the 96-well reading was performed using a Beckman-Coulter Cytoflex flow cytometer equipped with a plate reader. For each well, a minimum of 6,000 events was recorded. Since the beads are differentiated by size and internal fluorescence (FL4 channel), analyte-specific populations can be segregated using forward and side scatters properties followed by FL4 channel fluorescence analysis (each bead set has a

different FL4 intensity). The PE fluorescent signal quantified will be proportional to the concentration of tested adhesion molecules according to the standard curve generated in the same assay. The flow cytometry data were uploaded in FCS-3.0 file format on the LEGENDplex™ Cloud-based Data Analysis Software (Dec 05, 2019, BioLegend) for analyte quantification. Each sample included was tested in duplicate.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (v 8.1.1) (GraphPad Software, San Diego, CA, USA). Unadjusted associations of the variables listed above were assessed with Spearman's rank correlation, the nonparametric version of the Pearson correlation which reduces the influence of extreme values. The effect of age and global cognition on the correlation coefficients and *p*-values was calculated using the Partial and Semi-Partial (Part) Correlation ("ppcor") R package (v 1.1) [21]. Significance was set at $p < 0.05$ (two-tailed).

RESULTS

Demographic and clinical characteristics were as expected for this population with the exception for the low prevalence of hypertension (Table 1). Considering the broad range of age and cognitive performance of the cohort included in the present study, we first explored the association between age and global cognition with brain amyloidosis, blood levels of LPS, SCFAs, cytokines, and adhesion molecules (Supplementary Figure 1). Age was associated with the pro-inflammatory cytokines IL6 and TNFα and the adhesion molecules VCAM-1, CD44, and ICAM-3 ($0.22 \leq \text{rho} \leq 0.40$, $p < 0.046$). Global cognition, measured using MMSE and ADAS-cog, wan mainly associated with valerate, IL1β, CXCL2, and IL10, several adhesion molecules as well as with all the markers of brain amyloidosis ($0.25 \leq \text{rho} \leq 0.42$, $p < 0.040$).

Brain amyloid load is associated with circulating GMB-related products

In order to improve our understanding on the relationship between brain amyloid deposition and GMB, we measured the plasma levels of the Gram-negative membrane protein LPS and four SCFAs produced by the intestinal bacteria, namely acetate, propionate, valerate, and butyrate. Association of circulating GMB-related products with global and regional cortical amyloid load was evaluated using Spearman's rank test. Elevated levels of LPS were found associated with greater amyloid pathology in all the regions considered (Fig. 1). In particular, LPS resulted stronger associated with amyloid load in frontal, posterior, anterior cingulate cortex, and precuneus ($0.44 \leq \text{rho} \leq 0.49$, $p < 0.001$) than in temporal and parietal cortex (rho = 0.35 and 0.032, $p = 0.006$ and 0.011, respectively). Among the SCFAs, a positive relationship between acetate and valerate as well as a negative association of butyrate with brain amyloid deposition was observed (Fig. 1). High levels of acetate and butyrate were stronger associated with

Table 1
Demographic and clinical features of the 89 study participants

Socio-demographics	Mean (SD) or N (%)	Min-Max
Age (y)	69.6 (6.9)	54.1–86.0
Female	48 (54%)	n.a.
Education (y)	8.7 (4.1)	4.0–18.0
Body mass index[a]	25.3 (3.8)	17.0–37.1
Weight loss/gain	22 (25%)	n.a.
Florbetapir (standardized uptake value ratio)	1.11 (.22)	0.69–1.72
Cognitive performance		
Mini-Mental State Examination	22.8 (5.4)	2.00–30.0
ADAScog	16.2 (10.21)	4.0–55.6
Cognitive stage		
Unimpaired/ MCI/ dementia	16/39/34 (18/44/38%)	n.a.
Risk factors		
Hypertension	46 (52%)	n.a.
Diabetes	13 (15%)	n.a.
Vascular diseases	17 (19%)	n.a.
Hypercholesterolemia	39 (44%)	n.a.
History of stroke	7 (8%)	n.a.
Drugs		
Acetylcholinesterase inhibitors	6 (7%)	n.a.
Memantine	0	n.a.
Antidepressants/ hypnotics/ anxiolytics	34 (38%)	n.a.
Antipsychotics	5 (6%)	n.a.
Food supplements	11 (12%)	n.a.
Drug abuse	0	n.a.
Alcohol abuse[b]	3 (3%)	n.a.
Sleep patterns[c]	4.16 (4.80)	n.a.

[a]weight/height^2 and measured in kg/cm^2. ADAScog, Alzheimer's Disease Assessment Scale–Cognitive Subscale. [b]Alcohol abuse referred to the past, not currently in progress. [c]The following five night sleep symptoms were evaluated: difficulty falling asleep, waking up frequently in the night, early morning awakenings, not feeling rested in the morning, difficulty staying awake and need to take a nap during the day. For each symptom, respondents were asked to estimate frequency of the symptom during the past month on a five-point scale: 0 = never; 1 = 1–3 days; 2 = 4–7 days; 3 = 8–14 days; 4 = 15–21 days; 5 = 22–31 days [88].

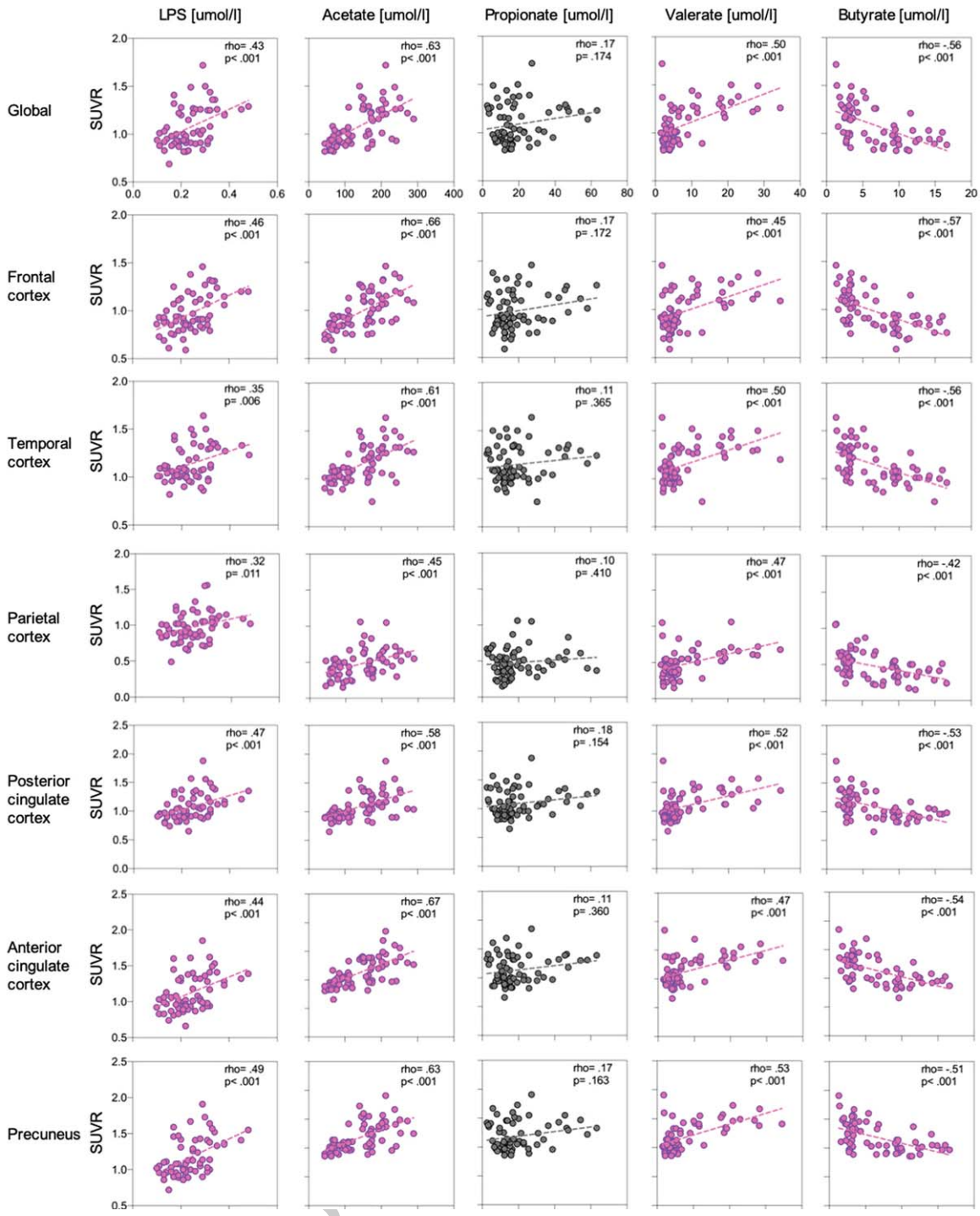

Fig. 1. Association matrix of circulating GMB-related products (LPS and SCFAs) with cortical amyloid load (SUVR). Spearman's rank correlation analyses included PET and SCFAs data for 67 subjects, PET and LPS data for 61 subjects. Two-tailed significant correlations are shown in purple. SUVR, standardized uptake value ratio of florbetapir.

frontal, temporal, posterior, anterior cingulate cortex, and precuneus ($0.58 \leq \text{rho} \leq 0.66$, $p < 0.001$ for acetate; $-0.57 \leq \text{rho} \leq -0.51$, $p < 0.001$ for butyrate) than parietal cortex ($\text{rho} = 0.45$, $p < 0.001$ for acetate and $\text{rho} = -0.42$, $p < 0.001$ for butyrate) while valerate did not show any region-specific association

($0.45 \leq \text{rho} \leq 0.53$, $p < 0.001$). In contrast, propionate was not associated with PET biomarkers ($0.07 \leq \text{rho} \leq 0.18$, $p \geq 0.182$). Similar findings were obtained after controlling the Spearman's Rank Correlations for age or global cognition measured using MMSE (Supplementary Figure 2).

Brain amyloid load is associated with circulating inflammatory mediators

Next, we evaluated the RNA gene expression from blood circulating cells of several circulating pro- and anti-inflammatory cytokines and we investigated their relationship with brain amyloid pathology. Elevated expression of 4 out of 7 pro-inflammatory cytokines, namely IL1β, NLRP3, IL6, and CXCL2 were associated with higher amyloid load at both global and regional level (Fig. 2). The stronger association between cortical amyloid deposition and inflammation was found in the posterior cingulate cortex ($0.38 \leq \text{rho} \leq 0.43$, $p < 0.001$) and the weaker association in the parietal cortex ($0.25 \leq \text{rho} \leq 0.35$, $0.001 \leq p \leq 0.023$). Among the anti-inflammatory cytokines, high expression of IL10 but not IL4, was found consistently associated with low amyloid deposition in all the regions considered ($-0.31 \leq \text{rho} \leq -0.26$, $p \leq 0.018$). Removing the outliers did not affect the correlation analysis between amyloid and IL1β, CXCL2, and IL10. Similar findings were obtained adjusting the Spearman's Rank Correlations for age or global cognition measured using MMSE (Supplementary Figure 3).

Circulating GMB products are weakly associated with peripheral inflammation

As both LPS and SCFAs are known to modulate inflammatory response, we tested the association between peripheral GMB-related products and inflammatory mediators. High levels of LPS, acetate and valerate were correlated with greater gene expression levels of at least one of the pro-inflammatory cytokines IL1β, NLRP3, CXCL2, and IL18 ($0.23 \leq \text{rho} \leq 0.35$, $p \leq 0.095$, Fig. 3). Moreover, high levels of acetate were weakly associated with lower expression of the anti-inflammatory cytokine IL10 ($\text{rho} = -0.24$, $p = 0.060$). Conversely, elevated butyrate levels were associated with lower levels of the pro-inflammatory NLRP3 and IL6 ($\text{rho} = -0.24$, $p = 0.060$ and $\text{rho} = -0.31$, $p = 0.016$, respectively) and of the anti-inflammatory IL4 ($\text{rho} = -0.24$, $p = 0.068$) as well as with higher levels of TNFα ($\text{rho} = 0.24$, $p = 0.066$). Propionate did not show any association with inflammatory biomarkers ($0.02 \leq \text{rho} \leq 0.21$, $p \geq 0.104$). Similar findings were obtained adjusting the Spearman's Rank Correlations for age but not for global cognition where p-values higher than 0.1 were reported for LPS and IL1β, valerate and IL18, and acetate and IL10 (Supplementary Figure 4).

Endothelial dysfunction is associated with circulating GMB products, inflammatory mediators, and brain amyloid load

To verify our hypothesis that GMB products and systemic inflammation compromise blood-tissue barrier integrity leading to vascular damage and AD pathogenesis, we measured the plasma levels of a panel of adhesion molecules. The upregulated expression of such molecules is one of the phenotypic features of endothelial dysfunction. Among the GMB products, the SCFAs acetate and valerate were found consistently associated with endothelial damage (Fig. 4). Indeed, elevated levels of acetate and valerate were moderate or strongly associated with high levels of almost all the endothelial markers considered ($0.25 \leq \text{rho} \leq 0.46$, $p \leq 0.045$). In contrast, butyrate seemed involved in the maintenance of the endothelial integrity, as suggested by its negative associations with NCAM, P-Selectin, and PSGL-1 ($-0.35 \leq \text{rho} \leq -0.25$, $p \leq 0.038$). LPS was weakly associated only with P-Selectin ($\text{rho} = 0.23$, $p = 0.099$).

Overall, elevated levels of adhesion molecules were associated with high expression of proinflammatory cytokines ($0.26 \leq \text{rho} \leq 0.42$, $p \leq 0.040$) and low expression of the anti-inflammatory IL10 ($-0.29 \leq \text{rho} \leq -0.26$, $p \leq 0.027$). IL4, usually considered an anti-inflammatory cytokine, showed a correlation profile similar to that identified for the pro-inflammatory cytokines as it was positively associated with 6 out of 13 adhesion molecules ($0.27 \leq \text{rho} \leq 0.40$, $p \leq 0.036$). Finally, elevated levels of CD44, NCAM, ICAM-2, P-Selectin, E-Selectin, PECAM-1, PSGL-1, ICAM-1, and ICAM-3 were associated with amyloid deposition in almost all the cortical regions considered ($0.25 \leq \text{rho} \leq 0.52$, $p \leq 0.042$). Similar findings were obtained adjusting the Spearman's Rank Correlations for age or global cognition measured using MMSE (Supplementary Figure 4).

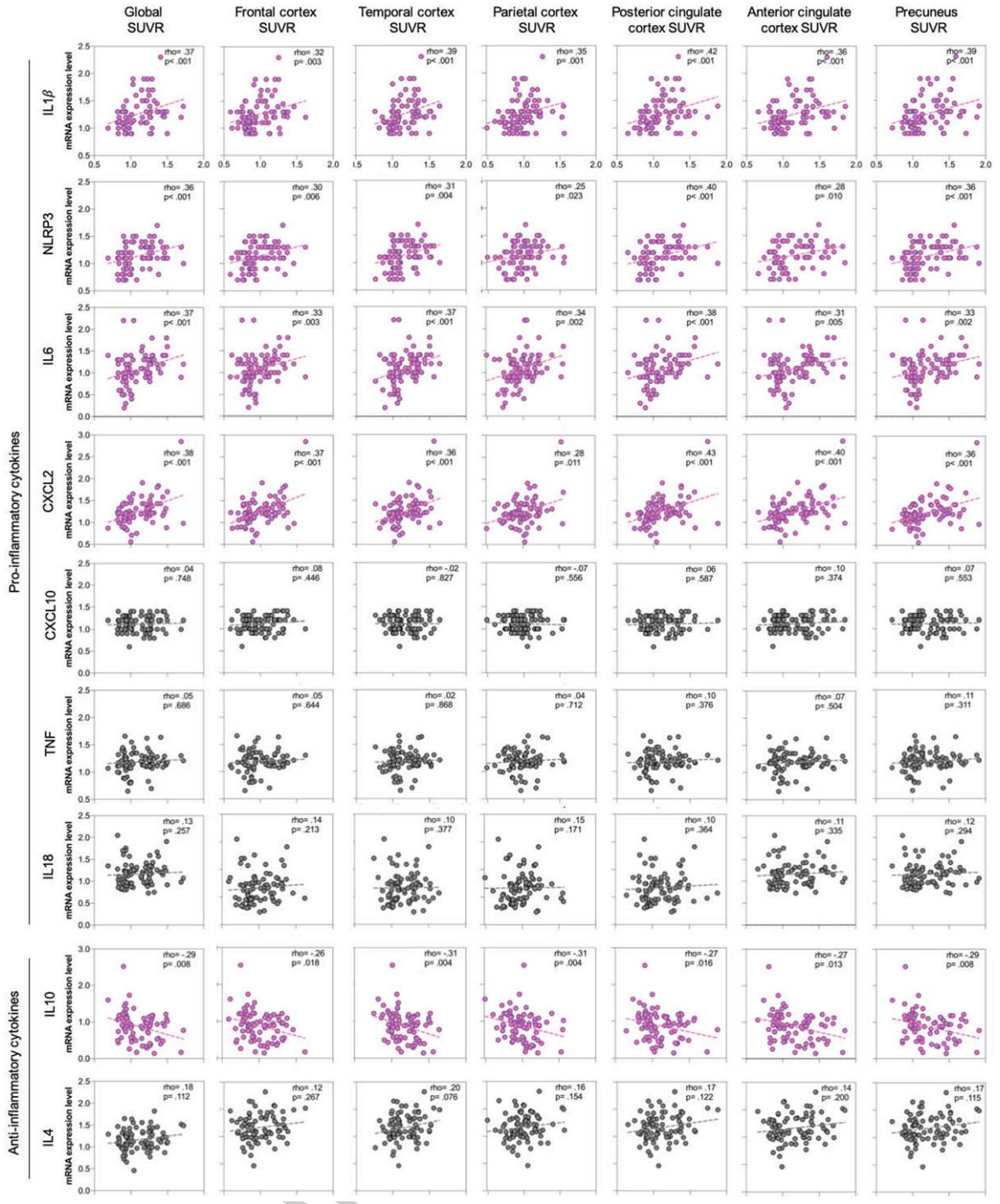

Fig. 2. Association matrix of circulating cytokines with cortical amyloid load. Spearman's rank correlation analyses included PET and inflammatory cytokine data for 83 subjects. Two-tailed significant correlations are reported in purple. CXCL2, Chemokine (C-X-C motif) ligand 2; IL, interleukin; NLRP3, NOD-, LRR- and pyrin domain-containing 3; SUVR, standardized uptake value ratio; TNF, tumor necrosis factor.

DISCUSSION

This is a cross-sectional study investigating the association among a number of circulating markers of GMB metabolism, systemic inflammation, and endothelial dysfunction, that might mediate the effect of the GMB on brain amyloidosis and thus be involved in the pathophysiology of AD (Fig. 5). The

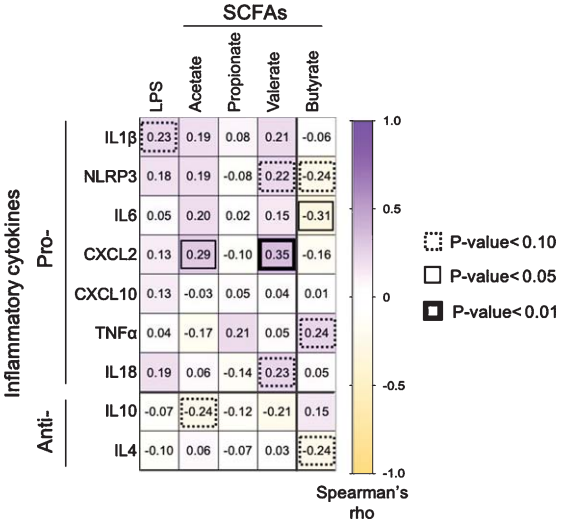

Fig. 3. Association matrix of circulating GMB-related products and cytokines. Columns represent GMB-related products; rows represent inflammatory mediators. Spearman's rank correlation analyses included data for 61 subjects. Figure denotes Spearman's rank correlation coefficient values. These are also represented on a yellow to purple color scale to facilitate the appreciation of association patterns. Significance is denoted by dotted and solid cell borders. CXCL2, Chemokine (C-X-C motif) ligand 2; IL, interleukin; LPS, lipopolysaccharide; NLRP3, NOD-, LRR- and pyrin domain-containing 3.

associations that we found confirm current knowledge on GMB metabolism and support the novel hypothesis of an effect of GMB-related products and systemic inflammation on brain amyloidosis, via endothelial dysfunction. Of course, the cross-sectional nature of our study prevents us to draw causal inferences. However, the strength of the associations (i.e., the size of the rho correlation coefficient) is coherent with the pathophysiological hypothesis.

Endothelial dysfunction in AD

The blood-tissue barrier is mediated by endothelial cell-cell adhesions as tight and adherents junctions as well as other CAMs, including PECAM-1 [22]. Several CAMs are also involved in leukocyte transendothelial migration by binding their leukocyte ligands and allowing activated leukocytes entry into the tissues [22]. Extracellular stimuli such as inflammatory mediators and SCFAs affect the expression of CAMs, influence the endothelial activation and thus, the blood-tissue permeability [23–25]. Increasing evidence suggests that several CAMs are involved in the pathophysiological processes of AD. However, the link of CAMs with microbiota and inflammation as a possible underlying mechanism of cerebral amyloid pathology has not been studied till now. *In vitro* studies demonstrated that Aβ and LPS increased the expression of ICAM-1 and the PECAM-1 [26, 27]. These molecules, found increased in the plasma AD patients [28, 29] and in AD plaques [30], mediate the transendothelial migration of leukocytes across the blood-brain barrier (BBB) [31, 32] and have been proposed to initiate the endothelial signaling cascade and influence the progression of neuroinflammation [32]. Increased levels of E-Selectin and P-selectin has been also reported in the plasma of AD patients [33, 34]. The former has been associated with vascular changes [34], the latter with fast cognitive decline [33]. Moreover, AD platelets show a significant increase in surface expression of P-selectin, that represents a marker of granule secretion [33, 35]. This pre-activated state of circulating platelets in AD patients has been proposed to contribute to dementia progression by triggering perivascular inflammation, induction of vasoconstriction, and consecutive brain hypoperfusion in addition to contributing to the peripheral Aβ pool [33]. The expression of the NCAM, considered an indicator of neurogenesis and neuronal plasticity, is altered in the brain and cerebrospinal fluid of AD patients compared with controls [36, 37]. The decrease of NCAM expression has been associated with cholinergic system alterations in the prefrontal and temporal cortex of humans [38] and inversely associated with phosphorylated tau, the other hallmark of AD pathology, in the murine entorhinal cortex [39]. *In vitro* studies using glioblastoma cells shown that CD44 induced neurodegeneration through the phosphorylation of tau [40] and attenuated the activation of the Hippo signaling pathway [41], recently found altered in several AD-related brain regions and proposed as an early event in AD development [42]. Furthermore, increased CD44 gene expression has been reported in lymphocytes derived from AD patients [43].

The effect of SCFAs and LPS on amyloid pathology: Direct and indirect via endothelial dysfunction and systemic inflammation

Direct evidences of the impact of SCFAs and LPS on amyloid aggregation come from *in vitro* and preclinical studies. Butyrate, but not acetate, inhibited the *in vitro* aggregation of soluble and neurotoxic $A\beta_{42}$ aggregates by interfering with the initial protein-protein interactions [14]. LPS potentiated the

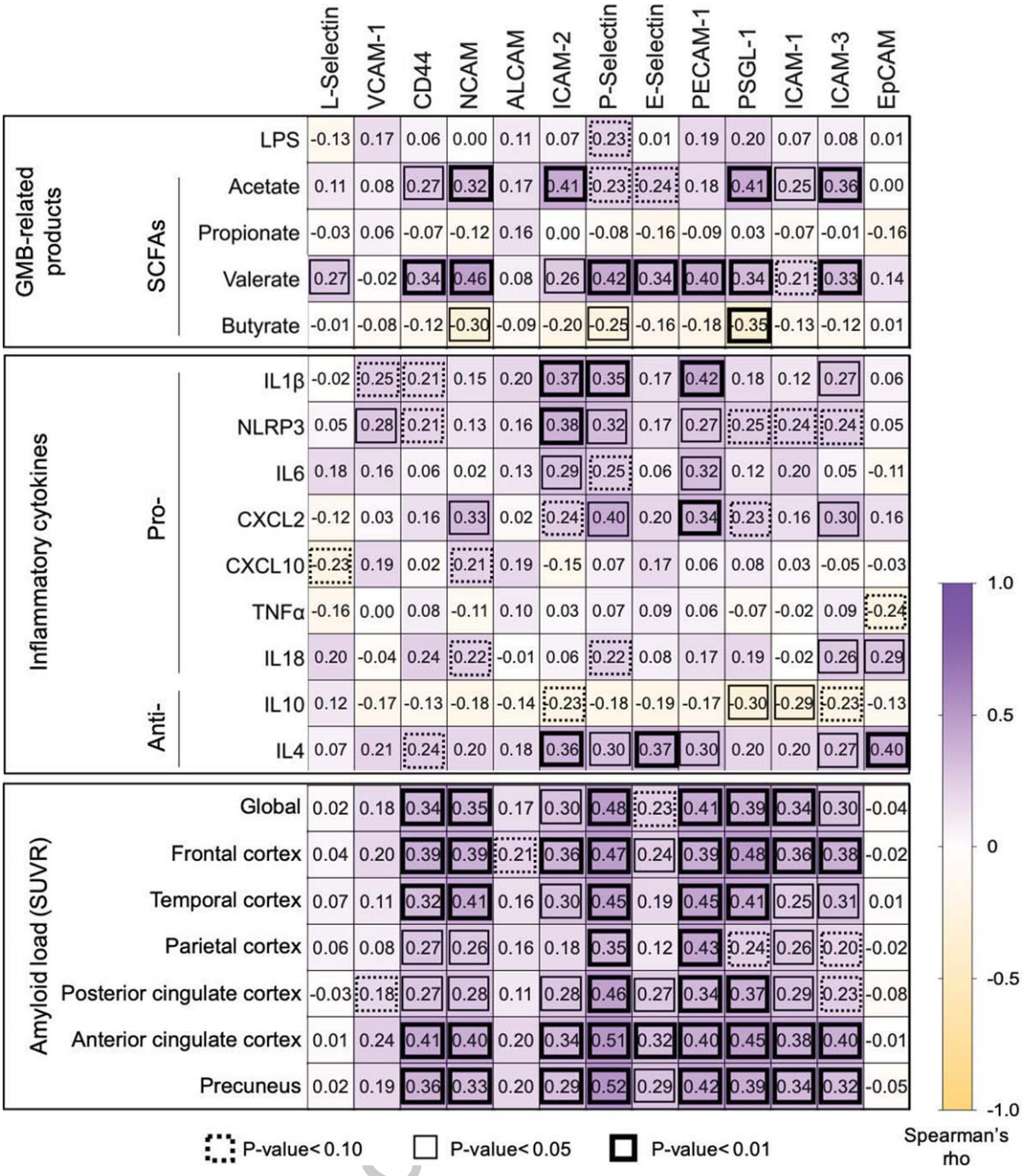

Fig. 4. Association matrix of circulating markers of endothelial dysfunction with circulating GMB-related products and cytokines and cortical amyloid load. Columns represent adhesion molecules; rows represent GMB-related products, inflammatory mediators and cortical SUVR uptake. Spearman's rank correlation analyses included data for 67 subjects for endothelial dysfunction markers, SCFAs and amyloid load, 61 subjects for endothelial dysfunction markers and cytokines, 52 subjects for endothelial dysfunction markers and LPS. Figures denote Spearman's rank correlation coefficient values. These are also represented on a yellow to purple color scale to facilitate the appreciation of association patterns. Significance is denoted by dotted and solid cell borders.

Aβ fibrils formation *in vitro* [44] and, when directly injected into the ventricles of rats, reproduced many of the inflammatory and pathological features seen in AD brains [45].

An indirect contribution of SCFAs and LPS on AD development via tissue-blood barrier impairment (gut-blood barrier (GBB) first and then the BBB) has been also proposed. Considering the gut

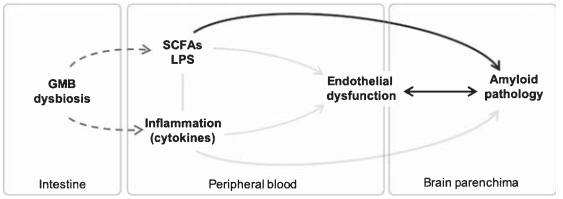

Fig. 5. Possible pathophysiological mechanisms for the GMB in AD. Straight arrow tones denote the strength of the association based on rho mean absolute values of the significant ($p < 0.05$) correlations: light grey, rho 0.30–0.35; dark grey, rho 0.4; black, rho > 0.5.

interface of the GBB, butyrate is important for maintaining the colonic epithelium. Indeed, it has been shown to enhance barrier function by supplying energy to intestinal epithelial cells and by increasing junctional integrity [46]. Conversely, LPS increased intestinal tight junction permeability [47]. At the brain interface of BBB, SCFAs, mainly butyrate, increased the expression of tight junction proteins in the frontal cortex and hippocampus of mice [48]. Systemic LPS and its components, known to be able to infiltrated the brain [49], induced BBB structural and functional alterations including the activation of the transcription of pro-inflammatory and cytotoxic pathways in astrocytes [50] and breakdown of inter-cellular tight junctions [51]. Considering the vascular interface of the barriers, butyrate acts as histone deacetylase inhibitor, transcriptional modulator, and anti-inflammatory molecule on microvascularity [52] and, conversely to acetate, decreased the endothelial Nlrp3 inflammasome assembly and IL1β production formation and activation in endothelial cells [53]. LPS activation of endothelia cells results in the production of various proinflammatory mediators, including IL6, and ultimately cellular injury [54]. Butyrate and LPS as well as acetate act as anti- and pro-inflammatory stimuli, respectively, also on innate and adaptive immune response. Butyrate promotes an antibacterial activity in intestinal macrophages and restricts bacterial translocation [55] while acetate mediates the production of cytokines (IL6, CXCL1, and CXCL2) by intestinal epithelial cells [56]. Butyrate ameliorated the pro-inflammatory response of immune cells to antigen stimulus as LPS by expanding the Treg cell populations [57] and by reducing the expression of pro-inflammatory cytokines (i.e., IL-6) in human mature dendritic cells [58].

The effect of cytokines on amyloid pathology: Direct and indirect via endothelial dysfunction

In addition to the pathways described above, cytokines might have a direct effect on amyloid aggregation or indirect via endothelial dysfunction. Increased local and systemic expression of cytokines have been reported in AD patients [59]. Cytokines as IL-1β and TNF-α are produced by microglia and astrocytes in response to exogenous and endogenous insults [60] or are selectively transported into the brain by the BBB [61]. Inflammatory mediators as IL1, IL6, IL18, TNF-α, and TGF-β1 affect AβPP expression level [62] and metabolism [63] possibly facilitating its amyloidogenic processing and increasing Aβ production and deposition. Moreover, chemokines produced in response to amyloid deposition, are responsible for the recruitment of peripheral immune cells, such as monocytes [64], neutrophils, and T cells [65], favoring the extent of local inflammation. These findings can be interpreted in the light of the emerging role of $A\beta_{42}$ as a physiological mediator of the innate immune system [1] and as an antimicrobial peptide with functions in the cerebral innate immune system [66]. Thus, its production in the brain may be a protective response to bacterial and viral infections [67]. In line, synthesized small-molecule inhibitors targeting *Porphyromonas gingivalis*, the keystone pathogen in chronic periodontitis identified in the brain of AD patients, reduced the brain bacterial load, blocked $A\beta_{42}$ production, reduced neuroinflammation, and rescued neurons in the hippocampus of infected mice [68]. Besides, it is also plausible that amyloid deposition in the brain is the results of age-related defects in the immune signals that monitor the molecular mechanisms implicated in its production, degradation, and clearance.

It is well known that BBB endothelial cells respond to inflammatory stimuli by generating vasoactive substances and through the modification of tight junction structures that increased barrier permeability [69]. Specific inflammatory mediators have been shown to be associated with the evolution of the BBB disruption [70]. Moreover, IL1β induced changes in BBB permeability [71] and the pathological relocation to the BBB of a chemokine (CXCL12) linked to leukocyte infiltration into the CNS [72]. IL-6 has been shown to disrupt the integrity of the BBB in rat brain endothelial cells [73], while NLRP3 deficiency ameliorated cerebral injury in mice after ischemic stroke by reducing infarcts and BBB damage [74].

The theory of inflammaging describes the low-grade, chronic, systemic inflammation in aging, in the absence of overt infection ("sterile" inflammation) [75]. While the features of inflammaging remain "normal" or "subclinical" in many elderly individuals, a portion of individuals (postulated to have a "high responder inflammatory genotype") may shift to age-associated diseases [76]. This shift has been proposed to serve as a prodrome or an exacerbating factor for development of AD [77]. Among the major identified sources of inflammaging, there are endogenous host-derived cell debris, amyloids, free radicals, immunosenescence as well as products and metabolites produced by the GMB [78]. Thus, our results fit well the hypothesis that poses inflammaging as the link between the gut microbiome alteration, that triggers and sustains systemic inflammation, and the inflammation leading the onset of the pathological hallmarks of AD [79].

This study has a number of limitations. First, we have not studied the effect of *APOE* genotype on the reported associations. The allele $\varepsilon 4$ of *APOE* is a known risk factor for brain amyloidosis and AD. While the effect of *APOE* genotype on GMB composition is unknown, the role of host's genome on the selection and growth of gut bacteria is well known [80], and might be responsible for some of the variance of the effects observed in the current study. Second, we have not taken into consideration oxidative stress, known to be contributes to endothelial damage [81–83], Aβ accumulation [84] and detected in the early stage of AD, even prior to the marked Aβ accumulation [85, 86]. Interestingly, GMB appears to be an electron acceptor and, by shaping the redox potential of the gut, influences the permeability of the intestine [87]. Third, the present study shares several aspects with a recent paper on the GMB in patients with AD [8]. However, differently from that work, here we have studied [^{18}F]-Florbetapir uptake as continuous variable, gaining in sensitivity, and included the evaluation of functional GMB-related variables such as LPS and SCFAs. Last, some of the studied markers were not available for all study participants. The research programs that contributed the data to the current study are still ongoing and recruiting participants, and future studies with a larger study population will allow to verify or falsify the current findings and also to evaluate other important biomarkers (i.e., *APOE* genotype, tau pathology, and oxidative stress biomarkers) not considered in the present manuscript.

In conclusion, we report a novel association of GMB-related products and systemic inflammation with brain amyloidosis possibly via endothelial dysfunction, suggesting that SCFAs and LPS represent candidate pathophysiologic links between the GMB and AD pathology. Reduction of butyrate together with increased acetate, valerate and LPS levels may compromise the tissue-blood barrier integrity, cause and sustain low-grade systemic inflammation and, ultimately, enter the CNS and facilitate the AD pathological cascade. Based on these evidences, examination of gut microbiome could present potential avenues and opportunities to discover and develop specific microbial signatures that are associate with the prognosis of AD progression. Ultimately, interventions that modulate the gut microbiome and enrich beneficial bacteria and bacterial metabolites may be helpful in ameliorating, preventing or slowing neurodegeneration.

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SUPPLEMENTARY MATERIAL

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REFERENCES

[1] Moir RD, Lathe R, Tanzi RE (2018) The antimicrobial protection hypothesis of Alzheimer's disease. *Alzheimers Dement* **14**, 1602-1614.

[2] Welling MM, Nabuurs RJA, van der Weerd L (2015) Potential role of antimicrobial peptides in the early onset of Alzheimer's disease. *Alzheimers Dement* **11**, 51-57.

[3] Minter MR, Zhang C, Leone V, Ringus DL, Zhang X, Oyler-Castrillo P, Musch MW, Liao F, Ward JF, Holtzman DM, Chang EB, Tanzi RE, Sisodia SS (2016) Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. *Sci Rep* **6**, 30028.

[4] Harach T, Marungruang N, Duthilleul N, Cheatham V, Mc Coy KD, Frisoni G, Neher JJ, Fåk F, Jucker M, Lasser T, Bolmont T (2017) Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. *Sci Rep* **8**, 7-41802.

[5] Bauerl C, Collado MC, Diaz Cuevas A, Vina J, Perez Martinez G (2018) Shifts in gut microbiota composition in an APP/PSS1 transgenic mouse model of Alzheimer's disease during lifespan. *Lett Appl Microbiol* **66**, 464-471.

[6] Zhuang Z-Q, Shen L-L, Li W-W, Fu X, Zeng F, Gui L, Lu Y, Cai M, Zhu C, Tan Y-L, Zheng P, Li H-Y, Zhu J, Zhou H-D, Bu X-L, Wang Y-J (2018) Gut microbiota is altered in patients with Alzheimer's disease. *J Alzheimers Dis* **63**, 1337-1346.

[7] Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, Carlsson CM, Asthana S, Zetterberg H, Blennow K, Bendlin BB, Rey FE (2017) Gut microbiome alterations in Alzheimer's disease. *Sci Rep* **7**, 13537.

[8] Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, Ferrari C, Guerra UP, Paghera B, Muscio C, Bianchetti A, Volta GD, Turla M, Cotelli MS, Gennuso M, Prelle A, Zanetti O, Lussignoli G, Mirabile D, Bellandi D, Gentile S, Belotti G, Villani D, Harach T, Bolmont T, Padovani A, Boccardi M, Frisoni GB (2017) Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol Aging* **49**, 60-68.

[9] Cahenzli J, Balmer ML, McCoy KD (2013) Microbial-immune cross-talk and regulation of the immune system. *Immunology* **138**, 12-22.

[10] Zhan X, Stamova B, Jin LW, Decarli C, Phinney B, Sharp FR (2016) Gram-negative bacterial molecules associate with Alzheimer disease pathology. *Neurology* **87**, 2324-2332.

[11] Zhan X, Stamova B, Sharp FR (2018) Lipopolysaccharide associates with amyloid plaques, neurons and oligodendrocytes in Alzheimer's disease brain: A review. *Front Aging Neurosci* **10**, 42.

[12] Scioli MG, Storti G, D'Amico F, Rodríguez Guzmán R, Centofanti F, Doldo E, Céspedes Miranda EM, Orlandi A (2020) Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: Potential diagnostic biomarkers and therapeutic targets. *J Clin Med* **9**, 1995.

[13] Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu K V, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva A V, Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG (2019) The microbiota-gut-brain axis. *Physiol Rev* **99**, 1877-2013.

[14] Ho L, Ono K, Tsuji M, Mazzola P, Singh R, Pasinetti GM (2018) Protective roles of intestinal microbiota derived short chain fatty acids in Alzheimer's disease-type beta-amyloid neuropathological mechanisms. *Expert Rev Neurother* **18**, 83-90.

[15] den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* **54**, 2325-2340.

[16] Zilberter Y, Zilberter M (2017) The vicious circle of hypometabolism in neurodegenerative diseases: Ways and mechanisms of metabolic correction. *J Neurosci Res* **95**, 2217-2235.

[17] Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, Schwierzeck V, Utermöhlen O, Chun E, Garrett WS, McCoy KD, Diefenbach A, Staeheli P, Stecher B, Amit I, Prinz M (2015) Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci* **18**, 965-977.

[18] Martins IJ, Fernando BWMAD (2014) High fibre diets and Alzheimer's disease. *Food Nutr Sci* **5**, 410-424.

[19] Boccardi M, Altomare D, Ferrari C, Festari C, Guerra UP, Paghera B, Pizzocaro C, Lussignoli G, Geroldi C, Zanetti O, Cotelli MS, Turla M, Borroni B, Rozzini L, Mirabile D, Defanti C, Gennuso M, Prelle A, Gentile S, Morandi A, Vollaro S, Volta GD, Bianchetti A, Conti MZ, Cappuccio M, Carbone P, Bellandi D, Abruzzi L, Bettoni L, Villani D, Raimondi MC, Lanari A, Ciccone A, Facchi E, Di Fazio I, Rozzini R, Boffelli S, Manzoni L, Salvi G Pietro, Cavaliere S, Belotti G, Avanzi S, Pasqualetti P, Muscio C, Padovani A, Frisoni GB (2016) Assessment of the incremental diagnostic value of Florbetapir F 18 imaging in patients with cognitive impairment: The Incremental Diagnostic Value of Amyloid PET With [18F]-Florbetapir (INDIA-FBP) Study. *JAMA Neurol* **73**, 1417-1424.

[20] Clark CM, Pontecorvo MJ, Beach TG, Bedell BJ, Coleman RE, Doraiswamy PM, Fleisher AS, Reiman EM, Sabbagh MN, Sadowsky CH, Schneider JA, Arora A, Carpenter AP, Flitter ML, Joshi AD, Krautkramer MJ, Lu M, Mintun MA, Skovronsky DM (2012) Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-beta plaques: A prospective cohort study. *Lancet Neurol* **11**, 669-678.

[21] Kim S (2015) ppcor: An R package for a fast calculation to semi-partial correlation coefficients. *Commun Stat Appl Methods* **22**, 665-674.

[22] Cerutti C, Ridley AJ (2017) Endothelial cell-cell adhesion and signaling. *Exp Cell Res* **358**, 31-38.

[23] Pober JS, Sessa WC (2007) Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol* **7**, 803-815.

[24] Li M, van Esch BCAM, Henricks PAJ, Garssen J, Folkerts G (2018) Time and concentration dependent effects of short chain fatty acids on lipopolysaccharide- or tumor necrosis factor α-induced endothelial activation. *Front Pharmacol* **9**, 233.

[25] Meijer K, de Vos P, Priebe MG (2010) Butyrate and other short-chain fatty acids as modulators of immunity: What relevance for health? *Curr Opin Clin Nutr Metab Care* **13**, 715-721.

[26] Giri R, Shen Y, Stins M, Du Yan S, Schmidt AM, Stern D, Kim KS, Zlokovic B, Kalra VK (2000) beta-amyloid-induced migration of monocytes across human brain endothelial cells involves RAGE and PECAM-1. *Am J Physiol Cell Physiol* **279**, C1772-1781.

[27] Shen Y, Sultana C, Arditi M, Kim KS, Kalra VK (1998) Endotoxin-induced migration of monocytes and PECAM-1 phosphorylation are abrogated by PAF receptor antagonists. *Am J Physiol* **275**, E479-486.

[28] Vestweber D (2015) How leukocytes cross the vascular endothelium. *Nat Rev Immunol* **15**, 692-704.

[29] Nielsen HM, Londos E, Minthon L, Janciauskiene SM (2007) Soluble adhesion molecules and angiotensin-converting enzyme in dementia. *Neurobiol Dis* **26**, 27-35.

[30] Verbeek MM, Otte-Höller I, Westphal JR, Wesseling P, Ruiter DJ, de Waal RM (1994) Accumulation of intercellular adhesion molecule-1 in senile plaques in brain tissue of patients with Alzheimer's disease. *Am J Pathol* **144**, 104-116.

[31] Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007) Getting to the site of inflammation: The leukocyte adhesion cascade updated. *Nat Rev Immunol* **7**, 678-689.

[32] Greenwood J, Etienne-Manneville S, Adamson P, Couraud PO (2002) Lymphocyte migration into the central nervous system: Implication of ICAM-1 signalling at the blood-brain barrier. *Vascul Pharmacol* **38**, 315-322.

[33] Stellos K, Panagiota V, Kögel A, Leyhe T, Gawaz M, Laske C (2010) Predictive value of platelet activation for the rate of cognitive decline in Alzheimer's disease patients. *J Cereb Blood Flow Metab* **30**, 1817-1820.

[34] Zuliani G, Cavalieri M, Galvani M, Passaro A, Munari MR, Bosi C, Zurlo A, Fellin R (2008) Markers of endothelial dysfunction in older subjects with late onset Alzheimer's disease or vascular dementia. *J Neurol Sci* **272**, 164-170.

[35] Laske C (2012) Clinical and biomarker changes in Alzheimer's disease. *N Engl J Med* **367**, 2050; author reply 2051-2.

[36] Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, Greenberg DA (2004) Increased hippocampal neurogenesis in Alzheimer's disease. *Proc Natl Acad Sci U S A* **101**, 343-347.

[37] Yin GN, Lee HW, Cho J-Y, Suk K (2009) Neuronal pentraxin receptor in cerebrospinal fluid as a potential biomarker for neurodegenerative diseases. *Brain Res* **1265**, 158-170.

[38] Aisa B, Gil-Bea FJ, Solas M, García-Alloza M, Chen CP, Lai MK, Francis PT, Ramírez MJ (2010) Altered NCAM expression associated with the cholinergic system in Alzheimer's disease. *J Alzheimers Dis* **20**, 659-668.

[39] Murray HC, Low VF, Swanson MEV, Dieriks BV, Turner C, Faull RLM, Curtis MA (2016) Distribution of PSA-NCAM in normal, Alzheimer's and Parkinson's disease human brain. *Neuroscience* **330**, 359-375.

[40] Lim S, Kim D, Ju S, Shin S, Cho I-J, Park S-H, Grailhe R, Lee C, Kim YK (2018) Glioblastoma-secreted soluble CD44 activates tau pathology in the brain. *Exp Mol Med* **50**, 5.

[41] Xu Y, Stamenkovic I, Yu Q (2010) CD44 attenuates activation of the hippo signaling pathway and is a prime therapeutic target for glioblastoma. *Cancer Res* **70**, 2455-2464.

[42] Xu J, Patassini S, Rustogi N, Riba-Garcia I, Hale BD, Phillips AM, Waldvogel H, Haines R, Bradbury P, Stevens A, Faull RLM, Dowsey AW, Cooper GJS, Unwin RD (2019) Regional protein expression in human Alzheimer's brain correlates with disease severity. *Commun Biol* **2**, 43.

[43] Uberti D, Cenini G, Bonini SA, Barcikowska M, Styczynska M, Szybinska A, Memo M (2010) Increased CD44 gene expression in lymphocytes derived from Alzheimer disease patients. *Neurodegener Dis* **7**, 143-147.

[44] Asti A, Gioglio L (2014) Can a bacterial endotoxin be a key factor in the kinetics of amyloid fibril formation? *J Alzheimers Dis* **39**, 169-179.

[45] Hauss-Wegrzyniak B, Vraniak PD, Wenk GL (2000) LPS-induced neuroinflammatory effects do not recover with time. *Neuroreport* **11**, 1759-1763.

[46] Mathewson ND, Jenq R, Mathew AV, Koenigsknecht M, Hanash A, Toubai T, Oravecz-Wilson K, Wu S-R, Sun Y, Rossi C, Fujiwara H, Byun J, Shono Y, Lindemans C, Calafiore M, Schmidt TM, Honda K, Young VB, Pennathur S, van den Brink M, Reddy P (2016) Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat Immunol* **17**, 505-513.

[47] Guo S, Al-Sadi R, Said HM, Ma TY (2013) Lipopolysaccharide causes an increase in intestinal tight junction permeability *in vitro* and *in vivo* by inducing enterocyte membrane expression and localization of TLR-4 and CD14. *Am J Pathol* **182**, 375-387.

[48] Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, Korecka A, Bakocevic N, Ng LG, Kundu P, Gulyas B, Halldin C, Hultenby K, Nilsson H, Hebert H, Volpe BT, Diamond B, Pettersson S (2014) The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* **6**, 263ra158.

[49] Vargas-Caraveo A, Sayd A, Maus SR, Caso JR, Madrigal JLM, García-Bueno B, Leza JC (2017) Lipopolysaccharide enters the rat brain by a lipoprotein-mediated transport mechanism in physiological conditions. *Sci Rep* **7**, 13113.

[50] Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. *J Neurosci* **32**, 6391-6410.

[51] Nau R, Sorgel F, Eiffert H (2010) Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. *Clin Microbiol Rev* **23**, 858-883.

[52] Chriett S, Dabek A, Wojtala M, Vidal H, Balcerczyk A, Pirola L (2019) Prominent action of butyrate over β-hydroxybutyrate as histone deacetylase inhibitor, transcriptional modulator and anti-inflammatory molecule. *Sci Rep* **9**, 742.

[53] Yuan X, Wang L, Bhat OM, Lohner H, Li PL (2018) Differential effects of short chain fatty acids on endothelial Nlrp3 inflammasome activation and neointima formation: Antioxidant action of butyrate. *Redox Biol* **16**, 21-31.

[54] Dauphinee SM, Karsan A (2006) Lipopolysaccharide signaling in endothelial cells. *Lab Investig* **86**, 9-22.

[55] Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F, Chomka A, Ilott NE, Johnston DGW, Pires E, McCullagh J, Sansom SN, Arancibia-Carcamo CV, Uhlig HH, Powrie F (2019) The short chain fatty acid butyrate imprints an antimicrobial program in macrophages. *Immunity* **50**, 432-445.e7.

[56] Kim MH, Kang SG, Park JH, Yanagisawa M, Kim CH (2013) Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **145**, 396-406.e1-10.

[57] Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **504**, 451-455.

[58] Nastasi C, Candela M, Bonefeld CM, Geisler C, Hansen M, Krejsgaard T, Biagi E, Andersen MH, Brigidi P, Ødum N, Litman T, Woetmann A (2015) The effect of short-chain fatty acids on human monocyte-derived dendritic cells. *Sci Rep* **5**, 16148.

[59] Brosseron F, Krauthausen M, Kummer M, Heneka MT (2014) Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: A comparative overview. *Mol Neurobiol* **50**, 534-544.

[60] Tuppo EE, Arias HR (2005) The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Biol* **37**, 289-305.
[61] Banks WA, Ortiz L, Plotkin SR, Kastin AJ (1991) Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. *J Pharmacol Exp Ther* **259**, 988-996.
[62] Forloni G, Demicheli F, Giorgi S, Bendotti C, Angeretti N (1992) Expression of amyloid precursor protein mRNAs in endothelial, neuronal and glial cells: Modulation by interleukin-1. *Brain Res Mol Brain Res* **16**, 128-134.
[63] Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, Hong JT (2008) Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. *J Neuroinflammation* **5**, 37.
[64] Fiala M, Zhang L, Gan X, Sherry B, Taub D, Graves MC, Hama S, Way D, Weinand M, Witte M, Lorton D, Kuo YM, Roher AE (1998) Amyloid-beta induces chemokine secretion and monocyte migration across a human blood–brain barrier model. *Mol Med* **4**, 480-489.
[65] Zenaro E, Pietronigro E, Della Bianca V, Piacentino G, Marongiu L, Budui S, Turano E, Rossi B, Angiari S, Dusi S, Montresor A, Carlucci T, Nanì S, Tosadori G, Calciano L, Catalucci D, Berton G, Bonetti B, Constantin G (2015) Neutrophils promote Alzheimer's disease-like pathology and cognitive decline via LFA-1 integrin. *Nat Med* **21**, 880-886.
[66] Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, Moir RD (2010) The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS One* **5**, e9505.
[67] Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, Washicosky KJ, Gyorgy B, Breakefield XO, Tanzi RE, Moir RD (2018) Alzheimer's disease-associated beta-amyloid is rapidly seeded by herpesviridae to protect against brain infection. *Neuron* **99**, 56-63.e3.
[68] Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, Nguyen M, Haditsch U, Raha D, Griffin C, Holsinger LJ, Arastu-Kapur S, Kaba S, Lee A, Ryder MI, Potempa B, Mydel P, Hellvard A, Adamowicz K, Hasturk H, Walker GD, Reynolds EC, Faull RLM, Curtis MA, Dragunow M, Potempa J (2019) Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Sci Adv* **5**, eaau3333.
[69] Liu Y-C, Tsai Y-H, Tang S-C, Liou H-C, Kang K-H, Liou H-H, Jeng J-S, Fu W-M (2018) Cytokine MIF enhances blood-brain barrier permeability: Impact for therapy in ischemic stroke. *Sci Rep* **8**, 743.
[70] Ott BR, Jones RN, Daiello LA, de la Monte SM, Stopa EG, Johanson CE, Denby C, Grammas P (2018) Blood-cerebrospinal fluid barrier gradients in mild cognitive impairment and Alzheimer's disease: Relationship to inflammatory cytokines and chemokines. *Front Aging Neurosci* **10**, 245.
[71] Blamire AM, Anthony DC, Rajagopalan B, Sibson NR, Perry VH, Styles P (2000) Interleukin-1beta -induced changes in blood-brain barrier permeability, apparent diffusion coefficient, and cerebral blood volume in the rat brain: A magnetic resonance study. *J Neurosci* **20**, 8153-8159.
[72] McCandless EE, Budde M, Lees JR, Dorsey D, Lyng E, Klein RS (2009) IL-1R signaling within the central nervous system regulates CXCL12 expression at the blood-brain barrier and disease severity during experimental autoimmune encephalomyelitis. *J Immunol* **183**, 613-620.
[73] de Vries HE, Blom-Roosemalen MC, van Oosten M, de Boer AG, van Berkel TJ, Breimer DD, Kuiper J (1996) The influence of cytokines on the integrity of the blood-brain barrier *in vitro*. *J Neuroimmunol* **64**, 37-43.
[74] Yang F, Wang Z, Wei X, Han H, Meng X, Zhang Y, Shi W, Li F, Xin T, Pang Q, Yi F (2014) NLRP3 deficiency ameliorates neurovascular damage in experimental ischemic stroke. *J Cereb Blood Flow Metab* **34**, 660-667.
[75] Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* **908**, 244-254.
[76] Giunta S (2006) Is inflammaging an auto[innate]immunity subclinical syndrome? *Immun Ageing* **3**, 12.
[77] Giunta B, Fernandez F, Nikolic WV, Obregon D, Rrapo E, Town T, Tan J (2008) Inflammaging as a prodrome to Alzheimer's disease. *J Neuroinflammation* **5**, 51.
[78] Franceschi C, Campisi J (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* **69 Suppl 1**, S4-9.
[79] Ising C, Venegas C, Zhang S, Scheiblich H, Schmidt S V, Vieira-Saecker A, Schwartz S, Albasset S, McManus RM, Tejera D, Griep A, Santarelli F, Brosseron F, Opitz S, Stunden J, Merten M, Kayed R, Golenbock DT, Blum D, Latz E, Buée L, Heneka MT (2019) NLRP3 inflammasome activation drives tau pathology. *Nature* **575**, 669-673.
[80] Blekhman R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, Spector TD, Keinan A, Ley RE, Gevers D, Clark AG (2015) Host genetic variation impacts microbiome composition across human body sites. *Genome Biol* **16**, 191.
[81] Chrissobolis S, Faraci FM (2008) The role of oxidative stress and NADPH oxidase in cerebrovascular disease. *Trends Mol Med* **14**, 495-502.
[82] Schreibelt G, Musters RJP, Reijerkerk A, de Groot LR, van der Pol SMA, Hendrikx EML, Döpp ED, Dijkstra CD, Drukarch B, de Vries HE (2006) Lipoic acid affects cellular migration into the central nervous system and stabilizes blood-brain barrier integrity. *J Immunol* **177**, 2630-2637.
[83] Haorah J, Ramirez SH, Schall K, Smith D, Pandya R, Persidsky Y (2007) Oxidative stress activates protein tyrosine kinase and matrix metalloproteinases leading to blood-brain barrier dysfunction. *J Neurochem* **101**, 566-576.
[84] Liu Z, Ren Z, Zhang J, Chuang C-C, Kandaswamy E, Zhou T, Zuo L (2018) Role of ROS and nutritional antioxidants in human diseases. *Front Physiol* **9**, 477.
[85] Zuo L, Prather ER, Stetskiv M, Garrison DE, Meade JR, Peace TI, Zhou T (2019) Inflammaging and oxidative stress in human diseases: From molecular mechanisms to novel treatments. *Int J Mol Sci* **20**, 4472.
[86] Smith MA, Hirai K, Hsiao K, Pappolla MA, Harris PL, Siedlak SL, Tabaton M, Perry G (1998) Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. *J Neurochem* **70**, 2212-2215.
[87] Reese AT, Cho EH, Klitzman B, Nichols SP, Wisniewski NA, Villa MM, Durand HK, Jiang S, Midani FS, Nimmagadda SN, O'Connell TM, Wright JP, Deshusses MA, David LA (2018) Antibiotic-induced changes in the microbiota disrupt redox dynamics in the gut. *Elife* **7**, e35987.
[88] Dello Buono M, Urciuoli O, De Leo D (1998) Quality of life and longevity: A study of centenarians. *Age Ageing* **27**, 207-216.