The impacts and influences of gut-microbiota dysbiosis and gut integrity on age-related neurological disorders.

A Report submitted as the examined component of the Project Module SXL390

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This work is dedicated to Toby, Leo and Lucy

who gave me the strength to get up on even the darkest mornings.

Abstract

As the global population becomes proportionately older, age-related neurological disorders such as Alzheimer's and Parkinson's Diseases present an increasing healthcare burden. Despite extensive research, the root causes of these diseases remain poorly understood, and therapeutic options often limited in their efficacy. Links with gastrointestinal factors have prompted research into the gut and its resident bacterial microbiota as potential contributory factors in initiation and progression of these diseases. In this review, current understanding of contributions of the gut microbiota and integrity of the gut environment to health are considered, examining the role of metabolite and gut related molecular messengers in communication with the brain via various biological routes of communication. Changes that take place to the relative balance between bacterial populations and the metabolites they produce with increasing ageing are seen to have an influence on integrity of the gut environment and these, together, on systemic and brain inflammation. Associations between increased relative abundance of pro-inflammatory molecules and Alzheimer's Disease are reported coupled with potential links with characteristic protein malfunction and reduced gut integrity. Dysbiosis in Parkinson's Disease is recognised in reduced abundance of anti-inflammatory butyrate producing species and raised inflammatory metabolite levels potentially linked with protein misfolding and pro-inflammatory conditions. Clearer understanding of gut influences on neurological disorders presents the opportunity for novel therapeutic approaches to treatment, including probiotic interventions and drug development aimed at gut microbiota and environment modification, and transfer of healthy gut microbiota between individuals. By furthering understanding of the influences of the gut on age-related neurological conditions, possibilities for earlier intervention and reduced progression of disease may potentially be developed.

Word count: 265

Abbreviations

5TH	Serotonin
Aβ	Amyloid-beta
AD	Alzheimer's Disease
α-syn	Alpha-synuclein
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
CNS	Central nervous system
CXCL2	C-X-C Motif Chemokine Ligand 2
ENA	Enteric nervous system
FMT	Faecal microbiota transplantation
GABA	Gamma-aminobutyric acid
GALT	Gut Associated Lymphoid Tissue
GF	Germ free
GI	Gastrointestinal
GM	Gut Microbiota
GMBA	Gut-microbiota-brain-axis
IgA	Immunoglobulin A (antibody)
IL	Interleukin
LPS	Lipopolysaccharide
MAMPs	Microorganism associated molecular patterns
NFT	Neurofibrillary tangles
NLRP3	NOD-like receptor protein 3
PD	Parkinsons Disease
ROS	Reactive oxygen species
SCFAs	Short chain fatty acids
TLRs	Toll-like receptors
TMA	Trimethylamine
TMAO	Trimethylamine-N-oxide
VN	Vagus nerve
WHO	World Health Organisation
XAN	Xanthoceraside
Xn	Xanthohumol

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1. Introduction

1.1 Background

Age-related neurological disorders represent a rising challenge as global populations become increasingly elderly, with the number of members of society aged over 60 predicted to double from approximately 1 billion to 2 billion in the period from 2020 to 2050, and those over 80 tripling in the same period (WHO, 2021a). Alzheimer's Disease (AD) accounts for 60-70% of global dementia, a condition predicted to affect 78 million people globally by 2030, with an estimated financial burden of US$ 2.8 trillion (WHO, 2021b). World Health Organisation (WHO) estimates of Parkinson's Disease (PD) in 2019 indicated that 8.5 million people were living with the condition globally, and that prevalence is seen to be increasing at a greater rate than any other neurological disorder (WHO, 2022). The causes of both diseases are complex and incompletely understood despite extensive and ongoing research, and an effective treatment for AD remains as yet elusive.

The community of bacteria resident in the gut, termed the gut microbiota (GM), is a major field of contemporary interest and research, with possible links to a wide range of diseases and disorders postulated and under investigation. Among these, associations between the risk factor of ageing, dysbiosis of GM and gut dysfunction have been described, and possible links proposed between the gut environment and development of AD and PD (Leite *et al.*, 2021), suggesting the potential of valuable additional understanding of contributory factors to, and therapeutic possibilities for, these complex, distressing and difficult to treat conditions.

1.2 Scope

This project critically reviews recent research (2016-2022) into impacts and influences of the GM and gut environment on the brain and central nervous system via pathways of the gut-microbiota-brain axis, and links between these and development of AD and PD. It is beyond the scope of this project to review GM links with other neurological diseases, or other risk factors associated with development of AD and PD. Whilst the key roles of inflammation and immune functions are considered in the above context, it is also beyond the scope of this review to cover aspects of these not associated with gut activity.

1.3 Study objectives:

- Outline links between age-related neurological disorders and gut microbiota and assess the impact on population health.
- Describe the pathways and mechanisms of communication between the gut and the brain, including the gut-microbiota-brain-axis and microbial metabolites.

- Outline the composition of healthy adult gut-microbiota and gut environment and the characteristic changes observed in ageing microbiota populations.
- Evaluate the evidence relating to gut microbiota dysbiosis on development and outcomes of the specified neurological disorders.
- Discuss potential prophylactic and therapeutic applications of gut microbiota modification.

1.4 Study methodology.

Literature searches were undertaken using Web of Science, Science Direct and PubMed databases, applying the following search terms:

- (gut brain axis) AND microbial metabolites
- (gut microbiota composition) AND changes AND ageing [exclude reviews]
- (healthy gut microbiota characteristic) [exclude reviews] [2018 – 2022]
- (gut microbiota eubiosis profile) [exclude reviews] 2018 – 2022]
- (gut microbial metabolite functions) [exclude reviews] [2018 – 2022]

Further papers were located from the reference lists of appropriate papers. Background information was obtained from known web sources or via Google search. All sources were evaluated for credibility by assessing relevance and timeliness, provenance, objectivity and methodology as per PROMPT analysis principles.

2 Gut microbiota, gut integrity and contributions to health

2.1 Gut microbiota and gut physiology

GM are complex populations of bacteria comprised primarily of Firmicutes and Bacteroidetes and a lesser proportion of Actinobacteria, Proteobacteria and Verrucomicrobia phyla. (Vascellari et al., 2020). The relative abundance of specific species between individuals (beta diversity) varies widely even among healthy cohorts, with distinct differences observed across geographic regions in a number of studies (Schirmer et al., 2016; Ueda et al., 2021). Presence of GM has been demonstrated to be key to development of physiological features of the immune system, such as Peyer's patches, Gut-Associated Lymphoid Tissue (GALT) and mucous layers (Spiljar, Merkler and Trajkovski, 2017), and healthy relative abundance of taxa is critical in maintaining a controlled balance of beneficial and pathogenic microbes (Holmes *et al.*, 2020).

Integrity of the gut environment is intrinsic to a healthy GM / host relationship. The luminal epithelium (Figure.1) of the intestine forms an effective barrier that prevents microbial translocation into the circulation, bound strongly by tight junction proteins (zonula occludens, occludin and

claudins) and adherens junction proteins (E-cadherin, β-catenin) (Spiljar, Merkler and Trajkovski, 2017; Shukla *et al.*, 2021). The epithelium features specialised cells, including goblet and Paneth cells that secrete mucin 2 and antimicrobial proteins that form a thick, protective, antibody rich mucous layer (Sovran *et al.*, 2019) which also serves as a source of energy for bacteria (Spiljar, Merkler and Trajkovski, 2017). Also present are polymodal enterochromaffin cells, expressing several specific receptors to monitor and respond to gut and GM activity via secretion of serotonin (5HT) to afferent nerve fibres (Bellono *et al.*, 2017). These collective features maintain equilibrium of the gut environment; disruption to any aspect of the barrier has the potential to compromise gut homeostasis (Shin and Kim, 2018).

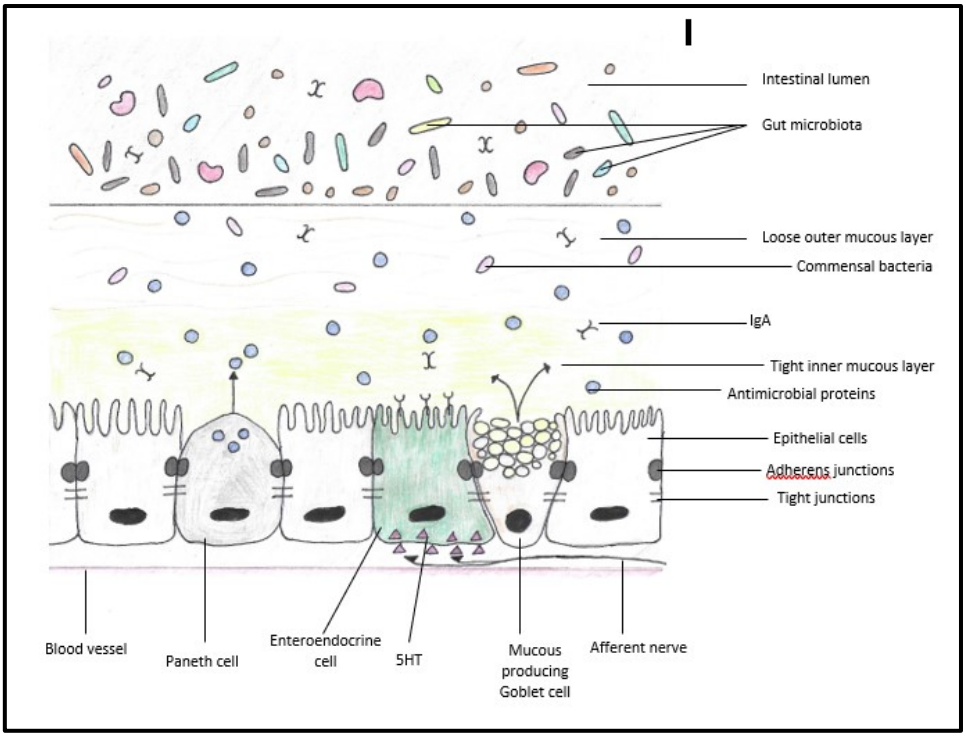

Figure 1. Protective features of the intestinal epithelium; inner (and outer - lower colon) mucous layers with antimicrobial proteins and IgA; specialised junctions and cells of the epithelium (author's interpretation).

2.2 Neuroactive metabolites and molecules of the gut

Fundamental to GM influence is the metabolism of dietary- and host-derived molecules and the products that result (Table.1). These range broadly in composition with wide and diverse functions and effects, both within the gut and the wider system beyond (Schirmer *et al.*, 2016; Kim *et al.*, 2020). Individual GM profiles determine the range, relative concentrations, and downstream pathways of metabolites present (Schirmer *et al.*, 2016; Lai *et al.*, 2021), playing a critical role in metabolism of carbohydrates and amino acids and conjugation of bile acids and neurotransmitters (Vogt et al., 2017, Lai et al., 2021). In addition, interaction between microorganism associated

molecular patterns (MAMPs) from bacteria, such as lipopolysaccharide (LPS), and host cells can have significant and wide-reaching effects on the system and brain (Spiljar, Merkler and Trajkovski, 2017; Vogt *et al.*, 2017; Kwon and Koh, 2020; Shukla *et al.*, 2021).

Table.1 AD/PD associated microbiota metabolites and gut molecules
Data from Schirmer et al., 2016; Spiljar, Merkler and Trajkovski, 2017; MahmoudianDehkordi et al., 2019; Srivastav et al., 2019; Doifode et al., 2021; Rosario et al., 2021; Bairamian et al., 2022; de Vos et al., 2022; Voigt et al., 2022

Neuroactive gut molecules	Metabolism:	Proposed functions:
SCFAs:		
Butyric acid	*Faecalibacterium prausnitzii*, *Eubacterium rectale*, *E. hallii, Ruminococcus bromii. Roseburia intestinalis, Butyrivibrio, Clostridia* spp,	Colonocyte energy source Intestinal tight junction regulation Reduces inflammation Regulatory peripheral t-cell production increases mucous secretion Promotes autophagy Increases acetylation of histone H3 Upregulates BDNF expression Neuroprotective effects
Acetic acid	Widely produced	Formation of lactate and gamma-aminobutyric acid (GABA) Regulation of lipid metabolism GM metabolised to butyrate
Propionic acid	*Akkermansia muciniphila*	Intestinal glucogenesis Regulation of lipid metabolism Contributes to lipogenesis Regulatory peripheral t-cell production
Non-SCFA:		
Tryptophan derivatives inc. serotonin (5-HT), melatonin, niacin, indole-3-ethanol, indole-3-pyruvate and indole-3-aldehyde	From dietary tryptophan via: 1. Kynurenine pathway (microbiota role) 2. Direct pathway 3. Serotonin pathway	1. Production of kynurenic acid, xanthurenic acid, picolinic acid, quinolinic acid, nicotinamide adenine dinucleotide – dysfunction associated with neurological disorders. 2. Production of indole and derivatives (directly by specific *Bacteroides* and *Clostridium* bacteria) – several anti-inflammatory, immunological and physiologically protective functions. 3. Production of 5-HT – multiple local and systemic functions. 4. Anti-Interferon (IFN)-γ effect
Secondary Bile acids inc. deoxycholic acid, lithocholic acid, ursodeoxycholic acid.	From bile acids (cholic acid, chendeoxycholic acid) via conjugation processes by GM.	Drug metabolism Gut immune regulation Pro-inflammatory – implicated in protective and destructive pathways.
Trimethylamine-N-oxide (TMAO)	Meta-organismal metabolism from high fat dietary micronutrients: to TMA by GM, then to TMAO by host enzymes.	Pro-inflammatory Risk factor in age related diseases May induce α-syn folding
MAMPS, PAMPS, DAMPS Inc lipopolysaccharide (LPS), peptidoglycan	Microbe / Pathogen / Damage Associated Molecular Patterns: bacterially derived molecules, e.g., cell wall of Gram-negative bacterium (LPS) recognised by host Pattern Recognition Receptors (PRRs) thereby initiating cascading immune and inflammatory responses.	

2.3 The Gut-Microbiota-Brain-Axis

The gut-microbiota-brain-axis (GMBA) describes the collection of bi-directional communication channels via which GM and metabolites interconnect with the central nervous system (CNS) and

brain (Lai *et al.*, 2021). GMBA descriptions vary between studies; here we consider three fundamental pathways (Figure.2):

1) *Neuroanatomical*. Principally via the vagus nerve (VN), this pathway represents a direct route of neural communication between the gastrointestinal (GI) tract and brainstem. The VN can be directly stimulated by cells of the gut, including enterochromaffin cells, via production of neuroactive metabolites and neurotransmitters (Bellono *et al.*, 2017; Kim *et al.*, 2019). In addition to typical nerve activation, Braak (2003) hypothesises that dysfunctional gut-derived amyloid proteins spread to the brain via the VN, however the exact mechanism of this remains unclear (Braak et al., 2003; Sampson et al., 2020; S. Kim et al., 2019)
2) *Circulatory*. Molecular access to the brain blood supply is tightly restricted by the blood brain barrier (BBB) of which the epithelia also comprise tight junctions, allowing passage and transport of only select molecules. Loss of integrity of tight junctions in the gut or BBB results in increased epithelial permeability ("leaky gut" and "leaky brain"), reduced control of molecule and microbe translocation and potential transit between the two loci. GM influence on the brain via the circulation has been observed experimentally in alteration in levels of oxidative stress, with wide ranging gut-derived molecules having both inflammatory and protective influences on the brain (Lai *et al.*, 2021).
3) *Immune*. Gut-based stimulation of immune responses following, for example, MAMP or IgA activation initiates cascades of cytokine production and complement activation, inducing activity in immune cells of the CNS. GM metabolites and gut molecules serve to increase or inhibit inflammatory immune responses (section 2.3.1), modifying systemic outcomes (Schirmer *et al.*, 2016; Spiljar, Merkler and Trajkovski, 2017; Shukla *et al.*, 2021).

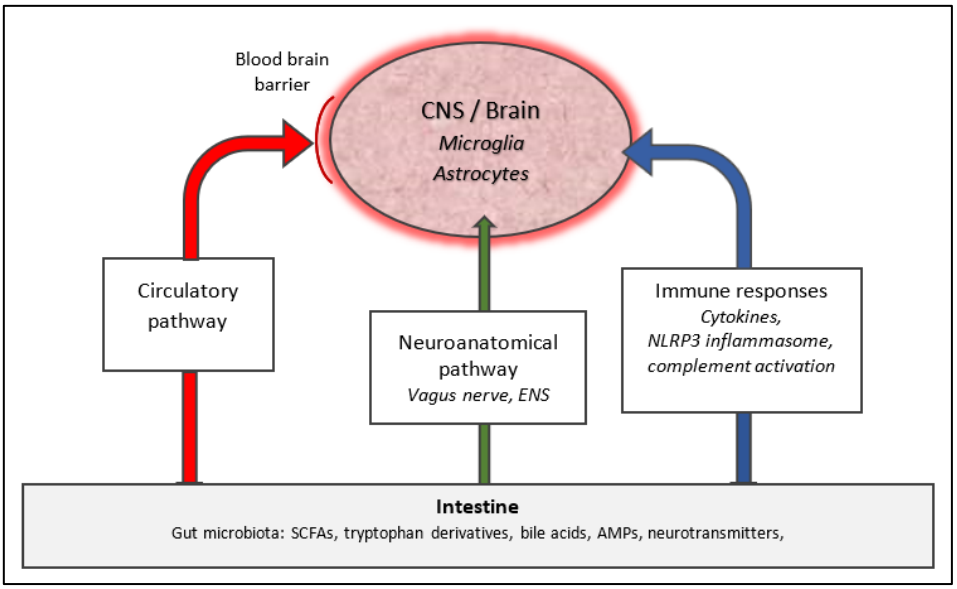

Figure.2 Modes of communication of the Gut-Microbiota-Brain-Axis (adapted from Nagpal et al., 2018; Doifode et al., 2021)

2.3.1 Immunity and inflammation

Immune and inflammatory responses are central to biological interactions between GM and host, primarily serving to identify and eliminate pathogenic threats. Immune stimulation, often following MAMP recognition by the Toll-like receptors (TLRs) expressed by immune cells, gives rise to cytokine production along with activation of further inflammatory factors such as the NLRP3 inflammasome, a key mechanism of innate nervous inflammatory responses (Kelley *et al.*, 2019; Shen *et al.*, 2020; Shukla *et al.*, 2021) (Table.2). When immune responses dysregulate, inflammation becomes disproportionate, inhibiting regenerative processes (Kwon and Koh, 2020; Rosario *et al.*, 2021). Persistent gut inflammation leads to increased permeability of intestinal and BBB epithelia, increased translocation of bacteria and molecules, and further inflammation. When chronic, these inflammatory interactions are strongly implicated in neurodegenerative disorder pathogenesis (Kelley *et al.*, 2019; Kwon and Koh, 2020; Leite *et al.*, 2021; Rosario *et al.*, 2021; Shukla *et al.*, 2021).

Considered to be of particular significance to neurodegenerative disorders is activation of microglia and astrocytes (glial cells), the principle immune cells of the CNS. Adapting in response to varying stimuli, glial cell phenotypes span a spectrum from neurotoxic (producing further pro-inflammatory mediators) to neuroprotective (producing anti-inflammatory mediators)(Kwon and Koh, 2020).

Table 2 The inflammatory nature of molecules (adapted from Bander 2020 and Cattaneo et al., 2017) Shukla,	
Pro-inflammatory	*Cytokines:* Interleukin (IL)-1β, IL-8, IL-12, IL-18, IL-23, Tumour necrosis factor-α (TNF-α) Monocyte Chemoattractant Protein-1 *Other molecules:* C-reactive protein (CRP) Lipopolysaccharide (LPS) C-X-C Motif Chemokine Ligand 2 (CXCL2) NLRP3 Inflammasome: NOD-like receptor protein 3 (NLRP3) associated with apoptosis-associated speck-like protein containing a caspase activating recruitment domain (ASC) and procaspase-1. Caspase-1 Gasdermin-D Trimethylamine-N-oxide (TMAO)
Anti-inflammatory	*Cytokines:* IL-10, IL-4, IL-27, IL-35 Transforming Growth Factor-β (TGF-β)
Variable	*Cytokines:* Interferon (IFN)-α, IL-6 *Other molecules:* SCFAs Bile acids / secondary bile acids

3 Gut changes and dysbiosis

3.1 Age-related gut and microbiota changes

Age-related immune decline is first observed in the mucosal immune system, with an overall increase in pro-inflammatory cytokine and NLRP3 protein production contributing to a state of chronic low-level inflammation (Sovran *et al.*, 2019; Shukla *et al.*, 2021). This inflammatory state – coined "inflamm-ageing"- is well described in association with age-related and neurodegenerative disorders, featuring increased reactive oxygen species (ROS) and exaggerated microglial activation (Franceschi *et al.*, 2018; Salazar *et al.*, 2019; Sovran *et al.*, 2019; Kwon and Koh, 2020; Leite *et al.*, 2021; Wu *et al.*, 2021).

Various murine studies demonstrate disordered and leaky gut epithelia in "aged" mice, with increased apoptosis of mucin 2 producing goblet cells resulting in a diminishing mucous layer and observably closer proximity of microbes to the intestinal epithelium in comparison to "young" mice (Sovran *et al.*, 2019; Wu *et al.*, 2021). Salazar *et al*.'s 2019 human study also describes decreased barrier function in elderly cohorts, citing links with significant reduction in SCFAs, possibly associated with dietary alterations and reduced fibre and polyphenol intake (Salazar *et al.*, 2019).

Findings of age-related GM changes vary between studies, some indicating increased GM diversity in the elderly (Wu *et al.*, 2020) and others a decrease (Sovran *et al.*, 2019). Differences in experimental design offer one possible explanation for these discrepancies. Salazar *et al.* describe significant positive correlation of *Akkermansia* and *Lactobacillus* taxa and significant negative correlation of *Faecalibacterium* taxa with increased age, but also acknowledge the inherent difficulties in drawing valid and unconfounded conclusions from an elderly human cohort (Salazar *et al.*, 2019).

Several studies into the impact of age on GM profile note distinctive and unexpected differences in those of centenarian cohorts (Sovran et al., 2019; Wu et al., 2020; Leite et al., 2021), including increased abundance of pro-inflammatory and pathogenic species (Sovran *et al.*, 2019; Bairamian *et al.*, 2022) suggesting a possible unique protective effect of some GM and metabolite profiles, or genetic response to them, supporting extension of healthy life into a second century.

3.2 Microbiota dysbiosis and gut dysfunction in Alzheimer's Disease

Alzheimer's Disease (AD) is characterised by accumulation of amyloid-beta (Aβ) protein and hyperphosphorylated tau protein into cerebral plaques and neurofibrillary tangles (NFT), the initiation of which is unknown, resulting in disrupted signalling and neuronal depletion (Honarpisheh

et al., 2020; Sampson *et al.*, 2020). Accumulations trigger pro-inflammatory and toxic microglial immune responses which become chronic, resulting in a spiralling cycle of pro-inflammatory molecule and ROS production, compromised phagocytic clearance, disturbed BBB, cell death, synaptic disfunction and neurological degeneration (Kwon and Koh, 2020; Shen *et al.*, 2020). Plaques have been identified in the gut of AD susceptible mouse models, with gut barrier disruption and disturbed cytokine responses reported prior to Aβ accumulation in the brain (Honarpisheh *et al.*, 2020), suggesting a possible early link between the gut and AD pathogenesis.

Several studies report significant differences between AD and control GM profiles, with some of these noting alterations occurring at pre-symptomatic stages (Vogt *et al.*, 2017; Kim *et al.*, 2020; Shukla *et al.*, 2021; Wang *et al.*, 2021). Exploration of the influences of GM on AD pathogenesis and progression using AD transgenic and/or germ free (GF) mouse models and faecal microbiota transplantation (FMT) add weight to this evidence, demonstrating increased plaque burden in transplants from AD donors, as well as improved cognitive function following transplants from non-AD controls to susceptible GF groups (Kim *et al.*, 2020; Wang *et al.*, 2021).

Increased abundance of *Escherichia/Shigella* species in AD is noted in multiple studies (Cattaneo *et al.*, 2017; Vogt *et al.*, 2017), with fragments of *Escherichia coli* (*E. coli*) and LPS reported in Aβ plaques and around AD cerebral blood vessels post-mortem (Zhan, 2016). Presence of *E. coli* is associated with several AD pathogenic markers, including production of pro-inflammatory cytokines interleukin (IL)-1β, NLRP3 and C-X-C Motif Chemokine Ligand 2 (CXCL2) (Cattaneo *et al.*, 2017), and altered bile acid ratios (Zhou *et al.*, 2022). Furthermore, *E. coli*-produced amyloid protein curli is associated with increased Aβ aggregation, potentially exacerbating disease progression in susceptible individuals (Sampson *et al.*, 2020).

Despite these observations, it is difficult to draw meaningful correlations relating to dysbiosis of specific bacterial families in AD from the research reviewed here, with multiple studies reporting contradictory findings of apparently significant increases and decreases in taxa. *Lactobacillus*, for example, is reported to be both increased (Honarpisheh *et al.*, 2020), and decreased (Shukla *et al.*, 2021) in susceptible subjects. Differences in mouse models (Tg2576 vs 5xFAD) and varying study objectives and methods offer two possible explanations for this significant discrepancy, and similar are noted widely within the pool of research informing this review.

Meanwhile, altered patterns in associated metabolites and molecules of the gut can be delineated in AD subjects. Decreases in SCFA levels and butyrate production are noted, as are levels of tryptophan and derivative 5HT (Cattaneo *et al.*, 2017; Zhou *et al.*, 2022), with correlation between changes in metabolism of bile acids to secondary bile acids - possibly reflecting altered GM enzymatic activity -

and cognitive impairment reported in several accounts (MahmoudianDehkordi *et al.*, 2019; Baloni *et al.*, 2020; Zhou *et al.*, 2022).

Additionally, increases in pro-inflammatory markers are widely described in association with AD models (Cattaneo *et al.*, 2017; Vogt *et al.*, 2017; Honarpisheh *et al.*, 2020; Kim *et al.*, 2020; Kwon and Koh, 2020; Shen *et al.*, 2020; Shukla *et al.*, 2021), with FMT modulation of the GM demonstrated to influence cytokine expression, microglia activation and upregulation of NLRP3 inflammasome (Kim *et al.*, 2020; Shen *et al.*, 2020). Furthermore, despite differences in research methods, several authors concur that evidence supports the early influence of neuroinflammation in development of AD. Increased serum inflammatory markers in association with circulatory LPS draw a potential link with impaired gut integrity (Kim *et al.*, 2020). This is supported by observation of reduced tight- and adherens junction protein expression and distribution in AD models, with correlating gut barrier breach (Honarpisheh *et al.*, 2020; Shukla *et al.*, 2021) and indication that altered barrier protein expression may occur at an early, pre-symptomatic stage (Honarpisheh *et al.*, 2020).

Finally, genetic alterations of colonic tissue observed in an AD mouse model, found to be associated with increased tissue degeneration and reduced mucosal immunity, were observed to be "normalised" following FMT from control littermates (Kim *et al.*, 2020), suggesting an intrinsic connection between GM and host gut integrity.

3.3 Microbiota dysbiosis and gut dysfunction in Parkinson's Disease

Parkinson's Disease (PD) is characterised by aggregation of misfolded amyloid protein alpha-synuclein (α-syn) in the substantia nigra and striatum brain regions, resulting in dopaminergic cell death, and is associated with loss of motor function and later cognitive decline (Sampson *et al.*, 2020; Simon, Tanner and Brundin, 2020). Pre-symptomatic enteric nervous system (ENS) disturbance (Sampson *et al.*, 2020), intestinal inflammation and gastric dysfunction (Vascellari *et al.*, 2020) support hypotheses that the disorder may have origins in the gut. Braak et al's influential 2003 work proposes proliferation of aberrant α-syn by recruitment and misfolding of endogenous proteins and spread in this prion-like way to the brain via the VN, an idea supported by experimental mitigation of α-syn aggregation following vagotomy (Sampson *et al.*, 2020). Bacterial-secretion derived curli formation is again found to be associated with this neurological disorder and demonstrated to exacerbate pathology in PD mouse models (Sampson *et al.*, 2020).

Dysbiosis is noted in several PD studies (Aho et al., 2019; Cattaneo et al., 2017; Vascellari et al., 2020; Voigt et al., 2022,), however specific taxa differences also vary considerably across this field of research. Mucin degrading species *Akkermansia muciniphila* is noted across several studies in

association with altered amino acids and bile acid production, however with contradictory reports of decreased (Huang *et al.*, 2019) and increased (Cirstea *et al.*, 2020; Vascellari *et al.*, 2020; Rosario *et al.*, 2021; Tan *et al.*, 2021) relative abundance in PD subjects. Despite both investigating PD GM changes in human faecal samples, two studies reviewed here reported substantially different taxa increases, from 10 species (Tan *et al.*, 2021) to 73 (Rosario *et al.*, 2021). Difference in sequencing techniques (16s rRNA vs shotgun metagenomics, respectively) and sample sizes offer two possible explanations for this variance. However, multiple studies agreed *E. coli* to be increased (Vascellari *et al.*, 2020; Rosario *et al.*, 2021) and associated with accelerated α-syn aggregation, increased inflammation and disease severity (Sampson *et al.*, 2020; Rosario *et al.*, 2021). Some agreement is also noted in findings of increased *Bifidobacterium* genera (Cirstea *et al.*, 2020; Vascellari *et al.*, 2020; Tan *et al.*, 2021), reductions in *Lachnospiraceae* (Huang *et al.*, 2019; Cirstea *et al.*, 2020; Vascellari *et al.*, 2020) and decreased *Prevotella* (Cattaneo *et al.*, 2017; Aho *et al.*, 2019; Rosario *et al.*, 2021).

In common with AD studies, more consensus is achieved when comparing metabolites than individual bacterial taxa, with reduced propionate and butyrate associated with decreased GM species, and links observed between decreased SFCAs and poor postural stability, constipation and reduced anti-inflammatory effects (Cirstea *et al.*, 2020; Rosario *et al.*, 2021; Tan *et al.*, 2021; Voigt *et al.*, 2022). Pathological and inflammatory improvements noted following increased butyrate levels, either directly or via probiotic intervention, further support the beneficial role of this metabolite in PD (Srivastav *et al.*, 2019; Voigt *et al.*, 2022).

A pro-inflammatory shift is broadly associated with PD, with toxic-state glial cell activation in the brain associated with degree of α-syn aggregation and presence of *E. coli* / MAMPs and observed to correlate with both gastrointestinal dysfunction and early-stage neural damage (Kwon and Koh, 2020; Sampson *et al.*, 2020; Voigt *et al.*, 2022). Furthermore, Rosario et al. postulate that reduced anti-inflammatory activity and increased systemic inflammation may contribute to initiation of α-syn aggregation and accumulation in the ENS, a notion supported by Voigt et al's finding of an α-syn folding effect of high concentrations of pro-inflammatory metabolite trimethylamine-N-oxide (TMAO) (Tables 1&2) (Rosario *et al.*, 2021; Voigt *et al.*, 2022). Shifts are also noted in a number of functional pathways in PD subjects, including increased methane metabolism, increased nucleic acid degradation and amino acid metabolism, reduced choline biosynthesis and bile acid degradation and reduced carbohydrate metabolism (Cirstea *et al.*, 2020; Rosario *et al.*, 2021; Tan *et al.*, 2021). Whilst Cirstea et al.'s observation that slowed gut transit rate may shift GM metabolism from carbohydrate to protein pathways, resulting in reduced butyrate generation, is a matter of interpretation, it may

be supported by findings of altered carbohydrate metabolism factors following probiotic therapy (Akbari *et al.*, 2016; Cirstea *et al.*, 2020).

Evidence relating to dietary impact on PD is contradictory across studies. Whilst Voigt et al's (2022) study indicates a strong correlation between consumption of foods rich in TMA precursors and PD, with reduced risk from the Mediterranean Diet, other studies report no significant correlation with dietary factors (Aho *et al.*, 2019; Vascellari *et al.*, 2020); sample size and differing study designs may again be influential in this regard.

The research into associations between GM and PD is recognized by several authors to lack consistent findings (Aho *et al.*, 2019; Vascellari *et al.*, 2020), with medication in particular noted to be a significant confounding factor in assessment of PD, in terms both of symptom control and in potential modification of GM and metabolite production (Aho *et al.*, 2019; Cirstea *et al.*, 2020; Vascellari *et al.*, 2020; Voigt *et al.*, 2022). Further sources of variance acknowledged include demographics, patient clinical details, technical protocols around sampling and storage and study design (Aho *et al.*, 2019), underlining the need for cautious interpretation when comparing study findings.

4 Therapeutic potentials of gut and microbiota modification in AD and PD.

4.1 Probiotic intervention

Probiotic bacteria are purported to have a beneficial impact on tight junction integrity, immune function and may help bolster the protective commensal community (Dore *et al.*, 2020; Mishra *et al.*, 2021). Susceptible mouse model studies indicate that pre-emptive probiotic treatment may mitigate glial cell activation and dopaminergic neuron loss and reduce PD symptoms, with a human trial finding significant reductions in systemic inflammatory C-reactive protein (CRP) (Table.2) and altered carbohydrate metabolism pathways, with significant improvement in cognitive measures reported (Akbari *et al.*, 2016; Srivastav *et al.*, 2019). Possible mechanisms of effect include raised butyrate levels in the brain, promoting neurogenesis and increasing neuroprotective Brain Derived Neurotrophic Factor (BDNF), suppression of which is also noted to be attenuated by probiotic treatment (Srivastav *et al.*, 2019). Although outside the scope of this review, probiotic alteration to insulin sensitivity is also associated with improvement of cognition in AD patients (Akbari *et al.*, 2016), presenting an interesting avenue of additional research. Despite some positive findings and an observed absence of side effects, limitations of research are noted. There is an acknowledged element of trial and error, with a lack of evidence relating to appropriate dosages (Akbari *et al.*, 2016) or effective combinations of probiotic strains (Srivastav *et al.*, 2019). Moreover, a

compromised gut barrier is seen to reduce the therapeutic effect of probiotics, instead resulting in an increase in microbe translocation and pro-inflammatory outcomes, potentially exacerbating rather than improving conditions. However, promising early findings of probiotic intervention warrant further research.

4.2 Drug development

Several compounds derived from plants are shown to have a beneficial effect on GM and subsequent improvements on ND. Xanthoceraside (XAN), a compound derived from husks of Xanthoceras sorbifolia, is seen to improve dysbiosis and has been found to have recovering effects on AD associated loss of SCFAs, amino acids, 5HT and gamma-aminobutyric acid (GABA). Study of XAN intervention demonstrates a reduction in AD related learning and memory loss in line with alterations to the GM. (Zhou *et al.*, 2022). Similarly, Xanthohumol (Xn) a flavonoid obtained from hops with many existing medicinal uses, increases GM in experimental AD model mice and is observed to suppress Aβ production and tau phosphorylation by regulation of amyloid precursor protein processing, with treated AD mice demonstrating improved performance in cognitive tests (Liu *et al.*, 2022).

Metformin, an existing drug found to be beneficial in treatment of PD (Rosario *et al.*, 2021), appears to promote improved mucin barriers of the gut, thereby reducing epithelial permeability and subsequent inflammation. Mechanisms of effect in this context are unclear but may include an increase in relative abundance of various bacterial species, raising levels of butyrate and taurine (Mishra *et al.*, 2021). Of interest for further investigation are links between GM, neurological disorders and metabolic disorders, for which Metformin is a commonly prescribed medication.

4.3 Faecal Microbiota Transplantation (FMT)

Along with frequently described use as an effective experimental device, FMT offers the potential to be deployed as a means of intervention for rectifying GM dysbiosis and is currently approved for use to treat chronic *Clostridium difficile* infection of the gut (NICE, 2014). Transplantation of GM from donor individuals to dysbiotic or germ-free models has been demonstrated to modify recipient GM, bringing it in line with that of the donor (Kim *et al.*, 2020; Wang *et al.*, 2021; Zhou *et al.*, 2022). Demonstrated effects of beneficial FMT GM modulation include reduction in system-wide inflammatory biomarkers such as CNS glial activation and intestinal expression of NLRP3 protein (Shen *et al.*, 2020), decreased soluble and insoluble amyloid in plasma with corresponding reductions in tau accumulation and plaque burden (Kim *et al.*, 2020) and alterations to metabolite profile, gut permeability and colonic gene expression (Kim *et al.*, 2020; Zhou *et al.*, 2022).

This experimental evidence may be supported by clinical case reports detailing profound improvements of cognitive (AD, PD) and motor (PD) function following FMT to treat chronic gut disorder and in trial as PD treatment. Such reports underscore the exciting therapeutic potential of this intervention for neurological disorder (Hazan, no date; Huang *et al.*, 2019), however, ethical considerations and the need for much further research are acknowledged before use of FMT intervention can be widened.

5 Discussion, future directions and conclusion

The body of research into the influences of GM on neurological disorders, of which only a small fraction is reviewed here, serves to illustrate the enormous complexity not only of the populations of microbes to which the gut is host but the countless molecular interactions and interconnections to which they contribute.

Much evidence indicates that GM has an influence on factors associated with the development, progression and symptoms of cognitive decline, as demonstrated by the effects of the GM manipulation in animal models described in this review (Srivastav *et al.*, 2019; Kim *et al.*, 2020; Sampson *et al.*, 2020; Shen *et al.*, 2020; Lai *et al.*, 2021; Shukla *et al.*, 2021; Wang *et al.*, 2021; Liu *et al.*, 2022; Zhou *et al.*, 2022). However, with the exception of a few repeated correlations, strong associations are not corroborated between specific taxa and development of neurological disorders, a fact widely acknowledged by authors of these works. Further complication comes with knowledge of the wide variation in GM communities of healthy individuals, including differences noted over geographical locations, raising the question of whether associations drawn between taxa and disease could be meaningfully extrapolated over wider populations.

Stronger correlations are noted here between systemic and CNS inflammation and declining cognitive health, associated both with the neurological disorders examined and more generally with older age (Cattaneo *et al.*, 2017; Kelley *et al.*, 2019; Honarpisheh *et al.*, 2020; Kwon and Koh, 2020; Sampson *et al.*, 2020; Shen *et al.*, 2020; Rosario *et al.*, 2021; Shukla *et al.*, 2021; Voigt *et al.*, 2022), this being mediated both by immune responses and directly by GM associated metabolites and molecules. Variation in the anti- and pro-inflammatory influences of gut molecules may be central to the impacts of GM, with dysbiotic populations resulting in imbalanced gut environment and loss of equilibrium. Whilst the importance of gut integrity in preventing GM and molecular translocation in control of wider systemic disruption is evident (Spiljar, Merkler and Trajkovski, 2017; Shin and Kim, 2018; Honarpisheh *et al.*, 2020; Rosario *et al.*, 2021; Shukla *et al.*, 2021), it is conceded that much

remains unknown of the complexity of interactions between GM and molecules of the gut and many questions remain.

A major limitation of this review in establishing connections between GM and neurological disorders is the magnitude of possible confounding factors when considering findings in a real-life context. The scale of potential extrinsic contributors to GM dysbiosis and gut equilibrium, such as diet, lifestyle, medication, comorbidities, exposure to pollution etc, accumulated over a lifetime, is unmeasurable.

Most particularly, the question of intrinsic genetic influences over all the factors associated with cognitive decline looms large and is an area underexplored in the literature reviewed here. Individual genetic variance is likely to impact every aspect of the processes discussed, from factors that influence aspects of ageing, such as cell regeneration and mitochondrial action (Kim *et al.*, 2020), to immune responses and inflammation, expression of tight junction proteins and production of elements that maintain the gut barrier (Sovran *et al.*, 2019), as well as how each host governs and interacts with its microbiota. A future direction of study into genetic susceptibility factors associated with influence of GM on neurological disorders may elucidate not only how the gut influences disease, but why some people are more affected than others.

In conclusion, links between GM profile and neurological disorders appear to be demonstrated by contemporary research with the potential to have profound significance for the increasing population of those at risk of their development. The vast complexity of individual GM profiles makes defining the characteristics of a healthy GM in anything but the broadest terms problematic and drawing firm conclusions as to the contributions of specific taxa to development of AD and PD is not achieved in the current literature. Changes to GM and the gut environment in later age are indicated, along with altered immune function and chronic low-level inflammation, however it is not possible to identify whether relationships between GM and these factors are causative or consequential. Evidence of the influence of bacterial metabolites and gut molecules on systemic inflammation, particularly in the context of a compromised gut environment, is of note. Associations can be drawn between immune responses to bacterial MAMPs and toxic inflammatory glial cell activation, acknowledged to correlate with neurological disorders, though a direct causal link cannot be assumed. Finally, several potential applications of GM modification for prevention or treatment of neurological disorders are identified and present worthwhile areas for future research into novel therapeutic approaches.

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