Identifying potential new opportunities for gut-brain mediating therapies in CNS diseases

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What is the gut-brain axis (GBA)?

The gut-brain axis (GBA) consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions. Recent advances in research have described the importance of gut microbiota in influencing these interactions. A next step is suggesting potential interventions on the gut microbiome to improve brain health and cure central nervous system related diseases (CNS) such as Alzheimer's Disease and Parkinson's Disease.

Already over 10 years ago evidence was available on the improvement of patients suffering from brain deterioration as results from elevated toxin levels in blood, by administration of oral antibiotics changing the gut microbiome. Furthermore, studies have shown that the level of dysbiosis of the gut microbiome is correlated to the level of autism in autistic patients.

In recent years research on the GBA has presented many working mechanisms coming from both preclinical work and clinical research involving IBS patients of which an overview is given below (Carabotti *et al.*, 2015):

From gut microbiota to brain:
Production, expression and turnover of neurotrasmitters (i.e. serotonin, GABA) and neurotrophic factor (BDNF)
Protection of intestinal barrier and tight junction integrity
Modulation of enteric sensory afferents
Bacterial metabolites
Mucosal immune regulation

Table 1: Excerpt of table by Carabotti showing potential working mechanisms from gut microbiota to brain.

This is further described by Sudo *et al.* (2004) that show data on microbial colonization of the gut in mice impacting the plasticity of neural regulation in response to microbiota. The article hypothesizes that components of the bacterial cell wall stimulate immune cells within the gut to release cytokines, which consequently influence the parts of the CNS involved in the regulation of the hypothalamic–pituitary–adrenal axis. Another animal study by Gareau *et al.* (2011) describes that a memory dysfunction occurs in mice showing bacterial infections as opposed to mice with a healthy microbiome. This effect is ascribed to an altered expression of brain-derived neurotrophic factor (BDNF), one of the most important factors involved in memory, caused by gut dysbiosis. Furthermore, De Vadder *et al.* (2018) identified "the mechanism of communication between the microbiota and enteric neurons as the initiation of serotonin release and subsequent activation of the 5-HT4 receptor".

Other neurotransmitters that are reported to play a role here are GABA, melatonin, histamine and biologically active peptides such as galanin. "These molecules propagate signals primarily through interaction with enteroendocrine cells (EECs), enterochromaffin cells (ECCs), and the mucosal

immune system, but some cross the intestinal barrier, enter systemic circulation, and may cross the blood-brain barrier". (Martin *et al.*, 2018)

Furthermore, microbiota affects the immune regulation of the intestinal mucosa by means of several mechanisms, such as the increase of substance P in the ENS, and the down-regulation of protease (Sinagra *et al.*, 2020). Proteases are enzymes that are upregulated in cases of gut dysbiosis and become the end-stage effectors of mucosal and enteric nervous damage.

Finally, a paper by Stilling (2014) suggest an epigenetic role that describes how ''some gut-microbial products can act as 'neuro-nucleomodulins' and thereby affect the epigenetic landscape of their host's brain cells which in turn has effects on host behaviour." Looking at available literature since 2014, the presented hypotheses have not been validated yet.

From this research work one can expect new microbiome related therapeutics are being developed. This prompted us to take a look at diseases related to the Central Nervous System (CNS) that are the most researched ones in relation to the gut microbiome. And also look at first leads that are being picked up towards clinical intervention trials.

Landscaping of research and clinical trials conducted in the gut-brain axis

In December 2020, we performed a text mining analysis making use of the TenWise KMAP (Knowledge Map) that contains over 200,000 biological relationships as described in medical literature. We applied a filter called 'CNS diseases' BioSet that contains the most relevant CNS diseases in terms of overall research conducted on these. Subsequently we searched with a selection of microbiome related terms (eg. microbiome, microbiota) in combination with each disease from the BioSet on all available literature abstracts in PubMed. We generated the following table that ranks (in order from large to small) the number of abstracts in which both the selected CNS disease (eg. anxiety) and the set of microbiome terms co-occur. A further look at sentence level enabled us to remove abstracts from this list that are clearly not relevant.

CNS disease	microbiome BioSet	# abstracts
alzheimer's disease	microbiome terms	634
anxiety	microbiome terms	586
parkinson's disease	microbiome terms	363
autism spectrum disorder	microbiome terms	339
schizophrenia	microbiome terms	169
mood disorder	microbiome terms	90
bipolar disorder	microbiome terms	72
amyotrophic lateral sclerosis	microbiome terms	58
epilepsy	microbiome terms	55
eating disorder	microbiome terms	42
acute stress disorder	microbiome terms	19
huntington's disease	microbiome terms	17

Table 2: Ranking of CNS diseases mostly related to the microbiome according to text mining conducted on relevant abstracts and titles.

This table shows clear pockets of GBA associated research based on the number of found abstracts that are indicators of significance of the research topic.

By reading the suggested abstracts from this table and by doing free text searches a further selection of abstracts was made by filtering for 'human clinical trials' and doing searches in the text on the microbiome related therapies: Fecal Microbiota Transplant (FMT), Bacteriophage therapy and Live Biotherapeutic Products (LBP) and its synonyms. *Prophylactic solutions such as probiotic and dietary interventions are not taken into account as focus here is on potential therapies*.

Finally, in order to get insights in unpublished results from recent human clinical trials we scanned clinicaltrials.gov.

The top 4: strong indications for the GBA: Alzheimer's, anxiety, Parkinson's and autism

Looking at relevant co-occurrences (number of abstracts in which both terms co-occur) the top 4 that has over 150 hits are: 1) Alzheimer's disease, 2) anxiety 3) Parkinson's disease and 4) autism spectrum disorder. It is apparent that a lot of research is existing linking the gut microbiome to these diseases. Within these publications, searching for the three different microbiome therapies: Fecal Microbiota Transplant, Bacteriophage therapy and Live Biotherapeutic Products (and its synonyms) have yielded hits with FMT: autism (24), anxiety (22), Parkinson's (20) and Alzheimer's (13) indicating that some active interventions studies in mainly animals with FMT are taking place. As a 2020 paper by Vendrik *et al.* mentions: 'A limited number of studies in humans have been performed or are ongoing, while for some disorders only animal experiments have been conducted. Large double-blinded randomized controlled trials are needed to further elucidate the effect of FMT in neurological disorders.'

Alzheimer's disease

The specific disease with the most hits with the microbiome is Alzheimer's disease (634 papers). This can be explained by its overall significance given the high amount of patients worldwide with an unmet medical need (35 million; 70% of all dementia patients) and its high growth rate. There are almost 4x more Alzheimer patients than there are Parkinson's patients.

Alzheimer's is a type of dementia that affects memory, thinking and behavior. Two abnormal structures called plaques (beta-amyloid) and tangles (tau) are prime suspects in damaging and killing nerve cells.

The possible modes of action of modulating the gut microbiome towards Alzheimer's Disease are described in the following papers:

Preclinical evidence/hypotheses

It is hypothesized that 'inflammatory microorganisms in gut microbiota are associated with peripheral inflammation and brain amyloid-β deposition in subjects with cognitive impairment. (Panza *et al.*, 2019).

However hardly any antibacterial therapies are being tested to date as it is unclear which constituents of the microbiome could be related to Alzheimer's. Some abstracts describe some gut bacteria being able to produce significant amounts of amyloids that might play a role in the eventual accumulation of amyloid in the brain. Also, often the role of the gut microbiota being able to produce neurotransmitters such as serotonin and GABA that play a positive in nerve signaling with special emphasis on the vagus nerve, is described.

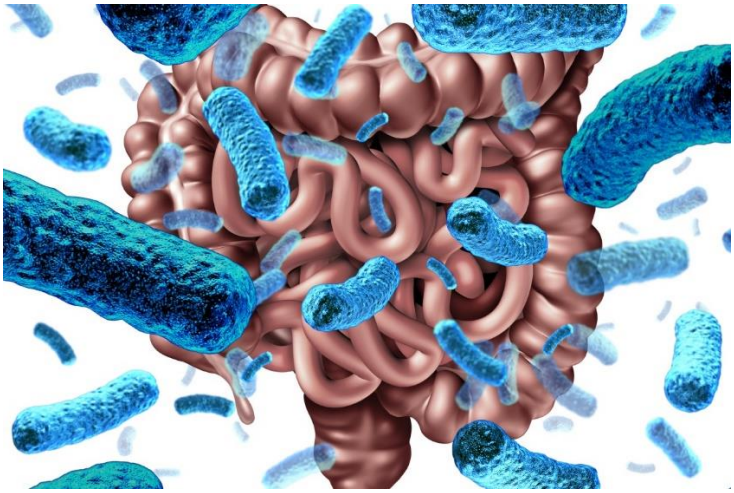

Figure 1: the gut microbiome is able to synthesise neurotransmitters such as serotonin that could be beneficial towards Alzheimer's Disease. Other constituents of the gut microbiome are however expected to be producing amyloids that could be linked to amyloid-β deposition in the brain, expected to cause Alzheimer's Disease.

Another hypothesis however is presented by Sochocka *et al.* (2018) with more focus on bacterial translocation:
"The increasing permeability of the intestinal mucosa and BBB (blood-brain barrier) in the elderly may intensify inflammatory reactions and induce amyloid aggregation" and "It seems that inflammatory-infectious hypothesis of AD, with the great role of the gut microbiome, starts to gently push into the shadow the amyloid cascade hypothesis that has dominated for decades."

This is supported by Li *et al.*, 2018: "It has been reported that AD brains contain higher bacterial levels than the brains of non-demented controls." and "The increase in BBB permeability seen during physiological aging may facilitate an increased rate of pathogen entry into the brain. This implies that age is a potential risk factor of Alzheimer's disease, and the intestinal microbiome may be responsible for pathogen infection in the brain and AD by changing the permeability of BBB."
This article also described the role of metabolites: "Metabolites produced by intestinal microbes are important molecules for neurological function. γ-Aminobutyric acid (GABA), the chief inhibitory neurotransmitter in the mammalian central nervous system, can be produced by *Lactobacillus* and *Bifidobacterium* species." Also described is the role of serotonin: "Intestinal microbiota play an important role in the synthesis of serotonin (5-hydroxytryptamine, 5-HT) which in turn plays a crucial role in the regulation of cognitive function."

Clinical interventions

Results in mouse and rat models are promising, however translatability to humans is still to be explored. Furthermore, interventions in humans are planned, but no results are reported to date. One, first anecdotal case describing positive results in an 82-year old male suffering from AD is described by Hazan (2020). The paper mentions a randomized, double-blind, placebo-controlled trial is currently underway to evaluate the efficacy of oral FMT in Alzheimer's disease. According to clinicaltrials.gov currently 10 clinical trials are underway on AD and the microbiome.

Anxiety (disorder)

Anxiety has a large number of hits (586 papers), but it needs to be noted that 'anxiety' is a general phenotype associated to mental health in general as it often mentioned together with 'depression', 'stress' and 'mood'. It can therefore be related to many, more specific CNS diseases and many other diseases that can cause a patient to develop anxiety.

The possible modes of action of modulating the gut microbiome towards anxiety are described in the following papers:

Preclinical evidence/hypotheses

Wong *et al.* (2016) conclude that "gut microbiota via inflammasome signaling modulate pathways that will alter brain function and affect depressive- and anxiety-like behaviors". Inflammasomes are cytosolic multiprotein oligomers of the innate immune system responsible for the activation of inflammatory responses.

Although the GBA-mechanism for anxiety disorder is unknown, Jiang *et al.* (2018) revealed: '…we found markedly decreased microbial richness and diversity, distinct metagenomic composition with reduced short-chain fatty acid (SCFA)-producing bacteria (associated with a healthy status) and overgrowth of bacteria, such as *Escherichia-Shigella*, *Fusobacterium* and *Ruminococcus gnavus*."

Another study performed in mice (Li *et al.*, 2019) using FMT yielded the following: "Interestingly, FMT affected both behavior and neuroinflammation. Mice given the CUMS microbiota had significant elevations of interferon-γ (IFN-γ) and the tumor necrosis factor-alpha (TNF-α) in the hippocampus, which were accompanied by upregulated indoleamine 2,3-dioxygenase 1 (IDO1) in the hippocampus. These results suggest that GM modulates pro-inflammatory cytokines in the hippocampus through dysfunctional microbiota-gut-brain axis, exacerbating anxiety- and depression-like phenotypes."

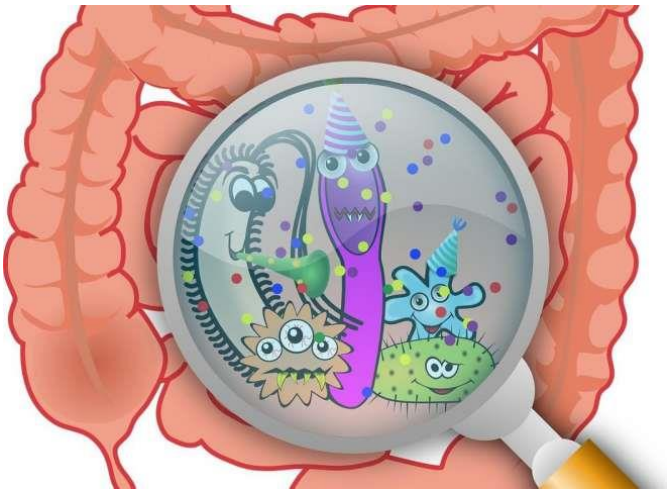

Figure 2: Overgrowth of certain bacteria of the gut microbiome such as *Escherichia-Shigella*, *Fusobacterium* and *Ruminococcus gnavus* have been suggested as beneficial microbes related to anxiety.

Clinical interventions

The research body describing clinical interventions for battling anxiety disorders is also limited: A review study by Arthi Chinna Meyyappan (2020) compares results of a.o. 8 clinical studies of which all show a positive impact on mental health after FMT with a healthy gut microbiome. It states: 'there appears to be strong evidence for the treatment and transmission of psychiatric illnesses through FMT. '

The same group from Queen's University, Canada describes a Phase 1 clinical trial with a live biotherapeutic product called MET-2 consisting of 40 gut derived strains that mimic a healthy gut microbiome and that was administrated to patient with general anxiety disorder and depression symptoms. The publication does not describe the results of the intervention. This study is sponsored by Nubiyota; a spinoff of the Canadian Mitacs.

Other human studies involving FMT investigated anxiety-related symptoms in subjects suffering from gut diseases such as IBS. Currently, 23 clinical trials are being conducted that are about 'anxiety' and the 'microbiome' according to clinicaltrials.gov.

Parkinson's disease

With 10 million patients worldwide Parkinson's disease (PD) is another large CNS disease with an unmet medical need that is being researched a lot in relation to the gut microbiome (363 papers).

Parkinson's disease is a progressive nervous system disorder that affects movement. Researchers have found that PD is associated to the formation of Lewy bodies in the brain and more specific the clumping of the protein alpha-synuclein inside these Lewy bodies.

Preclinical evidence/hypotheses

Gut microbiota have been studied in relation to the pathophysiology of Parkinson's disease (PD) due to the early gastrointestinal symptomatology and presence of alpha-synuclein pathology in the enteric nervous system, hypothesized to ascend via the vagal nerve to the central nervous system.

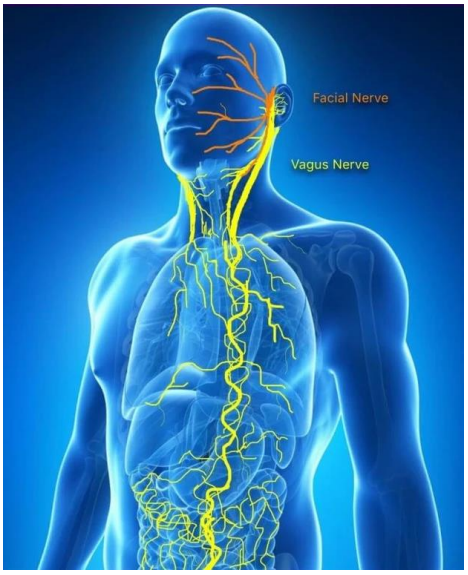

Figure 3: The vagus nerve links the gut with the brain. Braak hypothesized that abnormal alpha-synuclein can spread from the gut via the vagus nerve to the midbrain, where it selectively kills dopamine neurons, hereby progressing Parkinson's Disease.

Jackson *et al.* (2020) introduce and discuss some newer mechanistic explanations to the role of the gut microbiome: "Mechanistically this may be the consequence of changes in the relative abundance of SCFA-producing or LPS-containing bacteria in the intestinal microbiome with effects on intestinal barrier function, endotoxemia (i.e., systemic LPS), NLRP3 inflammasome activation, insulin resistance, and mitochondrial dysfunction, and the production of factors such as glucagon like peptide 1 (GLP-1) and brain derived neurotrophic factor (BDNF) as well as intestinal gluconeogenesis."

A large number of case controlled human studies have been conducted that show differences in gut microbiota between healthy groups and PD groups using the latest sequencing techniques. Almost all of these publications '…conclude that further studies are needed to determine whether the gut microbiota changes observed so far in PD patients is the cause or, instead, it is merely a consequence of lifestyle changes associated with the disease.' Keshavarzian *et al.*, 2020.

Clinical interventions

Unsurprisingly, human intervention studies with microbiome modulating therapies targeting PD are not published yet. However, according to clinicaltrials.gov 15 clinical studies are underway, most of these are carried out in the USA.

Autism Spectrum Disorder

One in each 60 children will develop a form of Autism Spectrum Disorder (ASD); boys being 4x more often diagnosed than girls. ASD is a complex neurological and developmental disorder characterized by behavioral and social impairments as well as multiple co-occurring conditions, such as gastrointestinal abnormalities, dental/periodontal diseases, and allergies. To date there is no cure. Suggested causes are gene variations and side-effects of drugs taken by the mother during pregnancy.
Body of research linking ASD with the gut microbiome is significant (339 articles).

Preclinical evidence/hypotheses

Vuong *et al.* (2018) indicate that "ASD patients also exhibited decreased *Bacteroidetes/Firmicutes* ratio, increased *Lactobacillus* and *Desulfovibrio* species, which correlated with ASD severity. ASD severity was also linked to a reduction in SCFAs, including acetate, proprionate and butyrate" but: "Despite these reports of microbial dysbiosis in ASD, there is little consensus on specific bacterial species that are similarly altered across separate studies. That is, no defined microbial signature has been identified for ASD, though many studies report microbiome differences within independent cohorts of ASD and controls."

Research by Sharon *et al.*, (2019) has shown that, next to genetics, the gut microbiome may play a role in the development of ASD: 'We transplanted gut microbiota from human donors with ASD or TD controls into germ-free mice and reveal that colonization with ASD microbiota is sufficient to induce hallmark autistic behaviors.'

Figure 4: Next to genetics, the gut microbiome may play a role in the development of Autistic Spectrum Disorder. ASD prevalence has increased with 178% since 2000 and in 2020 1 out of 54 children are diagnosed with ASD.

Clinical interventions

Nitschke *et al.* (2020) conclude after a systemic review of 19 studies that 'treatments such as special diets, vitamin, prebiotic, probiotic, and microbiota transfer therapy show promising therapeutic potential, yet are in their infancy of investigation.

Vendrik *et al.* (2020) mentions: 'Clinical trials with FMT have been performed in patients with autism spectrum disorder and showed beneficial effects on neurological symptoms.'
Currently 5 studies are underway that use FMT as intervention in ASD patients. These are conducted in USA and Israel (source: clinical trials.gov).

Potential upcoming areas within GBA: schizophrenia, mood disorder, bipolar disorder, ALS and epilepsy

The following CNS diseases display a limited amount of relevant hits: 5) schizophrenia, 6) mood disorder, 7) bipolar disorder, 8) amyotrophic lateral sclerosis and 9) epilepsy. This is an interesting group: there seems to be a reasonable amount of papers being published in recent years, so this could be a group of diseases that could be considered for therapy via microbiome modulation. A few intervention studies have been published on experimental FMT, notably in schizophrenia (7) and ALS (5). In the other indication no intervention studies have been published to date.

Preclinical evidence/hypotheses

A study by Evans *et al.* (2016) describes earlier work conducted "Schizophrenic and bipolar subjects show increased markers of bacterial translocation from the intestinal lumen (compared to controls), which may underlie increased inflammation known to be a component of these disorders. In depressed subjects compared to controls, a survey of the stool microbiota showed increased *Bacteroidetes, Proteobacteria* and *Actinobacteria* and reduced *Firmicutes Faecalibacterium*, the latter of which negatively associated with severity of depression." The results of the study by Evans indicate: "The most striking finding from the current study was the group difference and the clinical outcome associations for *Faecalibacterium* (OTU0003). *Faecalibacterium* is a Gram-positive butyrate-producing gut bacterium in large abundance in the human gut."

Diseases such as schizophrenia and bipolar disorder are being studied in relation to the microbiome but as stated by Chen *et al.* (2020): 'Given the dearth of current literature, more research is needed, however, to determine, which comes first in people with schizophrenia--an abnormal gut microbiota that elevates one's risk for schizophrenia or psychopharmacologic treatment of schizophrenia leading to secondary microbiota abnormalities or the negative symptoms of schizophrenia leading to obesity and its associated microbiota changes.'

Figure 5: Increased levels of *Bacteroidetes, Proteobacteria* and *Actinobacteria* and reduced *Firmicutes Faecalibacterium* are hypothesized to be correlated to reduced severity of depression, bipolar disorder and schizophrenia.

Clinical interventions

In December 2020 clinicaltrials.gov indicate the following number of clinical trials taking place: schizophrenia (3 recruiting), mood disorder (11 recruiting), bipolar disorder (3 recruiting, 2 completed), ALS (1 completed, 2 recruiting), epilepsy (3 recruiting, 1 completed). The vast majority of these studies are being undertaken in the USA.

Unlinked CNS diseases or yet to be 'discovered' relations with GBA

Hardly any (below 20 relevant publications per disease) hits with the microbiome were found with: eating disorder, acute stress disorder and Huntington's disease. This means that either there is hardly any evidence that links these diseases to the gut microbiome or that there has not been any research into that direction to date.

Preclinical evidence/hypotheses

Research includes mostly observatory studies on microbiome differences between healthy individuals and CNS patient groups and these diseases are often mentioned along with other CNS diseases indicating the potential of intervention via the microbiome.

Clinical interventions

Not surprisingly, no intervention studies on the gut microbiome have been published to date. The number of current clinical trials including microbiota analyses underway are 2 for eating disorders (anorexia nervosa).

Conclusions

- Text mining is a powerful and useful tool to find and rank CNS diseases based on preclinical and clinical research evidence related to the gut microbiome. Sentence analysis performed with our KMAP easily uncovers potential mechanisms of actions that play a role in health and disease and is helpful in removing non-relevant abstracts. This enables researchers to generate a comprehensive overview per disease within a matter of days instead of weeks.
- To date explorative studies are being undertaken displaying differences between microbiota of healthy groups and diseased groups. However, cause-effect relations are still largely unknown for most CNS diseases.
- From recent publications multiple mechanisms are hypothesized to play a role simultaneously in the gut-brain axis in relation to pathology of the most important CNS diseases. Described mechanisms include:
    - translocation of inflammatory bacteria via the blood-brain-barrier in conjunction with direct production of B-amyloids in Alzheimer's disease. In Parkinson's Disease abnormal alpha-synucleins are hypothesized to translocate via the vagus nerve to the brain;
    - an increase of gut microbiota that are able to modulate pro-inflammatory cytokines and microbial metabolites (SCFAs) that trigger immune response in the brain that relates to anxiety disorder, Parkinson's disease and autism spectrum disorder; and
    - an increase of gut microbiota that are able to produce or elevate levels of neurotransmitters such as serotonin and GABA in the main CNS diseases.
- Clinical interventions with predominantly Fecal Microbial Transplants (FMTs) and on rare occasions with Live Biotherapeutic Products (LBPs) and bacteriophage therapy, are currently taking place or are being considered are in the areas of:
    - **Alzheimer's disease**, **anxiety disorder**, **Parkinson's disease** and **autism spectrum disorder**. Currently, 15 microbiome-related clinical studies are underway related to Parkinson's Disease and 10 clinical studies related to Alzheimer's disease.
    - Potential upcoming CNS diseases that need further exploration in relation to the microbiome: **schizophrenia, mood disorder, bipolar disorder**, **ALS** and **epilepsy**. For all these diseases suggestions are made for interventions via the microbiome. Experimental FMT in animals has been applied in schizophrenia and ALS making these diseases likely the first targets for therapeutic development.