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Treatment of Autism spectrum disorder by targeting the gastrointestinal system

Thesis Behaviour & Neurosciences - WBBY901-05

Stijn van Eijk – S3737667
07-07-2021, Groningen

Supervised by prof. dr. A.J.W. Scheurink

Abstract

Autism spectrum disorder (ASD) is characterized by social and communicational difficulties, accompanied by restricted and repetitive behavior. This asks for suitable therapy options. ASD is tied to several comorbidities, one of them being gastrointestinal (GI) symptoms. ASD incidence is influenced both by genetics and environment. Current treatment options consist of both pharmacological and nonpharmacological interventions. Since ASD often co-occurs with gut disturbances, a new prospective for treatment can be found in the gut-brain axis (GBA). The GBA is a bidirectional communicational mechanism between gut and brain. It involves pathways through the nervous systems of the gut and the brain, neuroendocrine pathways, immunological pathways, and bacterial metabolite pathways. GI abnormalities like atypical metabolite production, increased gut permeability, increased serotonin production and maternal factors are hypothesized to have a contribution in ASD incidence. Possible ASD therapies that target GI issues are fecal microbiota transplantation (FMT), pro- and prebiotics and dietary interventions. Although positive effects have been reported for these therapies, effects were small, and extensive studies were limited. More research is necessary to establish if these treatments possibly can become conventional.

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects about 1 in 54 children in the US, roughly 2% of all children [3]. People who have ASD are known to have trouble in social interactions and communication; they have a harder time reading other people's emotions and may have difficulties building relationships with friends, having a romantic relation, or have difficulties fitting in and finding a suitable job.

What is surprising, is that people with ASD are also found to have problems in their digestive system. Some studies estimated that up to 84% of children with ASD have symptoms of gastrointestinal abnormalities [19]. This led to the idea that maybe, ASD could have a connection with the gut. In the last decade, this idea became more and more plausible.

'As recently as 2011, it was considered crazy to look for associations between the microbiome and behavior', stated in the New York Times, 2019. 'When the researchers investigated the microbiomes of these mice (with an ASD-like phenotype), they found the animals lacked a common species called *Lactobacillus reuteri*. When they added a strain of that bacteria to the diet, the animals became social again [1].'

This was the first sign that ASD behavior could be tied down to the mammalian gut. Apparently, there is a strong connection between the gastrointestinal system and the brain. Nowadays, a lot of research is done on this interaction, which is also called the gut-brain axis. About 2500 years ago, the Greek physician Hippocrates of Kos already said: 'All disease begins in the gut', and this inspires the research question of this thesis: 'How is ASD connected to the gut-brain axis and how can improving gut functioning help in ASD treatment?' To answer this question, ASD and the gut-brain axis will be thoroughly scrutinized.

Autism spectrum disorder

Autism spectrum disorder (ASD) is a heterogenous developmental disorder. ASD is a condition whereby patients typically show social difficulties, repetitive actions, and restricted interests. The way people with ASD communicate, behave, and interact is different than normal. Often, ASD develops within the first 2 years postpartum and can be diagnosed reliably from this age on [2]. In the U.S., approximately 1 in 54 children is diagnosed with ASD. Of this ratio, 1 in 34 are boys, and 1 in 144 are girls; boys are four times more likely to get the diagnosis ASD compared to girls. Autism affects every socioeconomic and ethnic group around the globe [3]. ASD does not include autism only, nowadays also Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS), and Asperger Syndrome are part of ASD [4].

The American Psychiatric Association's Diagnostic and Statistical Manual, Fifth Edition (DSM-5) is the diagnostic and statistical manual for all types of psychiatric disorders. The DSM-5 provides diagnostic criteria to determine ASD. The most important criteria are [4]:

- Deficits in social interaction and communication. This manifests in three ways:
    1. Deficits in social-emotional reciprocity, e.g., abnormalities in conversations, like disabilities to initiate and respond to conversations.
    2. Deficits in nonverbal communicative behavior used for social interaction, e.g., abnormalities in eye contact or facial expressions.
    3. Deficits in developing, maintaining, and understanding relationships, e.g., difficulties to make friends.
- Signs of restricted, repetitive behavior, interests, or activities. This manifests in at least two of the following:
    1. Stereotyped/repetitive movements, speech, use of objects.
    2. The need for ritualized patterns, inflexibility to changes.
    3. Interests that are restricted and fixated, and abnormal in intensity.
    4. Hyper-/hyporeactivity to certain aspects of sensory information, e.g., fascination for specific sounds or sights.

Even though concise criteria are provided by the DSM-5, there seem to be sex differences in the presentation of ASD. Males have shown to externalize their behavioral problems, like showing aggressive behavior, hyperactivity, decreased level of prosocial behavior, and higher levels of repetitive and or restricted behaviors and interests. On the other hand, females show higher levels of internalizing behavioral problems, including anxiety and depression symptoms, and other emotional symptoms. This is also a possible explanation for the four times higher more common prevalence of ASD for boys; the externalization behavior patterns are more disruptive and subsequently are easier observed and recognized in comparison to the internalization behavioral problems of girls [5].

Individuals with ASD are observed to have abnormal brain connectivity. In general, the connectivity seems to be more randomly organized, and often involves the frontal and occipital lobes. Atypical lateralization patterns - the activity balance between the two hemispheres - are also observed, in which the left hemisphere is functionally more active. General activity trends for ASD indicate overall long distance underconnectivity in comparison to neurotypical people, but also show overconnectivity in local areas. This could mean that ASD individuals have reduced connections in their long-range networks but can perform better at a selection of specific tasks [6].

Comorbidities of ASD

ASD has been found to be connected to several co-morbid medical conditions. Next to the fact that these comorbidities alone give rise extra challenges for individuals with ASD, it is also an increasingly more important topic of investigation since reducing these comorbid symptoms might also influence ASD severity. A few comorbidities are known to be of frequent occurrence when diagnosed with ASD.

Seizures

A significant amount of people with ASD have been known to eventually develop epilepsy. It has been found that the highest risk of epilepsy in ASD is for individuals with an intellectual disability, with a prevalence of 21%, while also the rate without intellectual disability is about 8%. This can be a variety of epilepsy types. It has been hypothesized that this comorbidity of ASD and seizures is due to a shared pathophysiology of their neurobiology [7].

Sleep disorders

There is a high prevalence of sleep disturbances among ASD individuals, ranging in rates between 40-80%. Abnormal patterns currently reported include later sleep onset, reduced sleep maintenance and poor sleep duration. These symptoms are more often observed in developing children but can stay more prolonged. One of the promising biological explanations for this co-occurrence is the role of melatonin, the sleeping hormone. It has been hypothesized that a dysregulation in the internal day and night rhythm (circadian rhythm) could lead to abnormal melatonin synthesis in ASD [7].

Psychiatric disorders

The co-occurrence of psychiatric disorders and ASD is also observed in up to 70% of people with ASD. The most common issue is anxiety, but also ADHD and oppositional defiant disorder (ODD). A wide variety of anxiety types is observed for ASD individuals, and often persist from childhood into adolescence [8].

Eating & gastrointestinal disorders

As earlier mentioned, gastrointestinal (GI) symptoms are known to have a high prevalence among ASD individuals, with rates climbing as high as 84% [19]. Children with ASD are up to five times more likely than neurotypical children to develop feeding issues, for instance high food selectivity. The type of observed food selectivity is mainly manifested as a carbohydrate and processed food preference. These feeding problems can last past childhood and into adulthood. GI symptoms are observed more often already in toddlers with ASD than without ASD and this could imply that there is abnormal gut development and functioning in ASD compared to neurotypical and non-ASD special needs populations. Recent data reveals that ASD children are four times more likely to have GI issues, with the most prevalent GI symptoms being constipation, diarrhea, and abdominal pain. The prevalence of GI issues is also proportionate to ASD severity [8].

Causes of ASD

Genetics of ASD

The etiology of ASD is strongly influenced by a genetic component. In the 1970s, it was already discovered in twin studies that identical twins had a 64% concordance in ASD incidence, while fraternal twins only had a 9% concordance. A large-scale research in Sweden (2014) proposed a 50% heritability for ASD. All in all, genetics have an important role in ASD development [8]. A broad heterogenous collection of mutant genes are found to be a

culprit for ASD development; the etiology of ASD is almost always multigenic – a combination of multiple abnormal genes – and every case of ASD is a sum of multiple abnormal genes that together enhance ASD susceptibility. Most of the abnormal proteins formed by ASD risk genes can be classified into two groups [9].

First off are the synapse formation proteins. The synapse is the location where one neuron transmits its electrical signal to the adjacent neuron, via the release of neurotransmitters. The dysfunction of parts of this system leads to synapse pathology and an abnormal neural network, which has been observed for ASD [9].

Second are the transcription regulation/chromatin-remodeling proteins. These proteins are involved in transcription; the process of converting DNA into mRNA. Chromatin-remodeling proteins are involved in modulating the shape and compactness of DNA; chromatin is a collection of packaged DNA and regulatory proteins. Mutations in proteins for these systems are mostly observed *de novo*, meaning that these are new mutations that are not inherited from the parents but arise from mutations in the egg cell of the mother, or the sperm cell of the father [9].

All in all, since a disruption of only a small part of the whole system can cause an imbalance in the complete regulatory processes described above, hundreds of different types of gene mutations can lead to somewhat the same presentation of ASD, making it genetically very diverse. To exemplify, the most frequently observed gene that is disrupted in ASD is called CHD8 - a chromatin regulator gene involved in fetal development – but such variants are only found in approximately 0,5% of children with ASD [10]. Therefore, it is impossible to appoint one type of mutations as the culprit of ASD.

Another genetic factor that contributes to susceptibility of ASD are copy number variations (CNVs). CNVs are structural variants in chromosomes, like variants with duplications, deletions, translocations, and inversions. These CNVs can both arise *de novo* or be inherited. Estimates suggest that CNVs are involved in around 10% of ASD cases. Only the more frequently occurring CNVs have been studied so far, such as 16p11.2 duplications. Having this CNV means that chromosome 16 has a duplicated piece of information on the location 11.2, making the chromosome a tiny bit larger than normal. Duplications of this part of the chromosome are thought to dysregulate synaptic transmission, causing abnormal communication between neurons. But again, also ASD-associated CNVs are rare and even the most frequently occurring CNVs, like the 16p11.2 variant, are only present in about 1% of ASD cases [9].

Environmental components
Aside from genetics only, the environment is essential as well, especially since these two factors interact with each other. For example, a mother prenatally exposed to harmful contaminants may cause genetic mutations in her baby, triggering ASD [11].
There are numerous types of environmental factors that could possibly contribute, and can be subdivided in a few categories [12]:

- Natural environment factors. This influences the early (neuronal) development process and includes air pollution such as long-term ozone exposure, exposure to environmental heavy metals like lead and mercury, organic toxicants like endocrine-disrupting chemicals (EDCs) which interfere with hormonal balance, pesticides which

target the nervous system, phthalic acids from cosmetics which causes inhibited testosterone production and seasonal factors like influenza outbreaks.
- Social and familial environment factors. During development, social factors also have an influence on ASD risk, and includes psychological stress during pregnancy, maternal migration (also stress related), or birth order in which later-born children have higher ASD incidence.
- Nutritional factors. An abnormal maternal metabolism during the pregnancy may be linked to ASD. Nutritional factors include (omega-3) fatty acids which are important for brain development, vitamin D which has key roles in the differentiation of neurons and promoting brain development, folic acid which is important for cell mitosis, and mineral deficiencies like zinc and magnesium which also inhibit neurodevelopment.

Concluding, environment and genetics both have an interconnected role in the risk of developing ASD during fetal development and early life. Interventions can intervene in this process and help reduce the risk of developing ASD or help improving the symptoms that are connected to ASD.

Therapies for ASD

Therapy is desired for ASD patients to improve social and cognitive functioning. The current types of therapy that are used for ASD can be subdivided in two types, pharmacological interventions, and nonpharmacological interventions. Since ASD is such a heterogenous spectrum with lots of variants, the therapy options are quite varied as well. As such, the main therapy options will be covered.

Pharmacological interventions

The main drug that is used for ASD treatment and the currently most promising medication option in ASD treatment will be discussed.

Risperidone is an antipsychotic drug which is the most widely used drug for ASD treatment [13], while it is mainly used to treat types of schizophrenia [14]. In ASD, it significantly reduces repetitiveness, aggression, anxiety, and nervousness. In a study with ASD children, 57% reduction in irritability versus 14% in a placebo group was observed. However, although these effects are positive, risperidone has been associated with adverse effects like substantial increases of appetite and subsequent weight gain, fatigue, drowsiness, and dizziness. These side effects make admitting risperidone to ASD patients a difficult decision, especially to children since the sleepiness effects can have adverse effects on school and other behavioral therapies [13]. These disadvantages induce a need for more suitable options.

Currently, a promising type of medication for ASD treatment are oxytocin and vasopressin, two closely related hormones differing only two amino acids in molecular structure. Receptors for these compounds have a high expression pattern in the amygdala (important for emotional regulation), hippocampus (important for cognition), and nucleus accumbens (important in the reward system) and are associated with social behavior, bonding, and parental care. Studies reported lower plasma oxytocin levels for ASD individuals, indicating deficits in its synthesis, which raised interest in the hormone as a possible therapy. Later research revealed that acute administration of oxytocin reduced repetitiveness and improved social cognition. Next to this, repeated administration showed benefits for ASD, with improvements in ASD social deficits in both children and adults. However, a different study showed that some side effects occurred due to peripheral effects of the hormone aside from

the effects in the brain, but these effects did not differ significantly from a placebo group. Vasopressin as an ASD treatment option improves reciprocity in communicative behavior, may enhance pro-social behavior and exert antidepressant and anxiolytic effects. Vasopressin has even been named the Breakthrough Therapy Designation in 2018 by the Food and Drug Administration (FDA) in the USA [13]. All things considered, these two hormones might become the first drugs to target the core social symptoms for ASD.

Nonpharmacological interventions
There are quite a few types of interventions that can help in treating ASD that does not involve taking medicine. The most important types will be covered.

Music therapy utilizes the ability of music to change the structure and neural connectivity of the cortex, resulting in enhanced multisensory integration which normally is impaired for ASD individuals. Passive music listening and actively making music both have proven to activate brain areas associated to cognition, sensorimotor, and perception brain regions because of heightened synchronization of the cortical areas. Music engages multisensory and motor networks and induces changes and new connections between distal but functionally related regions. Long-term practice in instrument playing has shown increases in the corpus callosum - the connective neural bridge between the right and left hemisphere – and in frontal, temporal, and motor areas of the brain [13].

Cognitive behavioral therapy (CBT) is called a psychotherapeutic therapy that is also used for depression, anxiety, and OCD. The goal of this intervention is psychoeducation; to change the thought patterns of the treated individual by teaching them their distortions in thinking and create better understanding of their own behavior and that of other people [15]. It mainly targets the core social symptoms of ASD, and by coaching, instruction, and providing structured worksheets, CBT addresses these social symptoms to improve this behavior. CBT has proven to be significantly effective in improving affective behavior, social skills, cognition, and facial emotion perception in ASD children and adolescents.

Social behavior therapy (SBT) teaches ASD individuals about functional independence and quality of life, by trying to improve communication, social skills, and emotional regulation. These interventions are often based on Applied Behavioral Analysis (ABA), an approach with the philosophy that environment is key for development of behavior. These therapy programs are therefore often used for early behavior in children. There is a broad variety of SBT programs that are comprehensive or specifically targeted at certain behavior [13].

Since ASD often has a comorbidity with GI system disorders, new treatment options are also emerging that target this system, which can be administered in various ways. The GI system might be an important target since the gut is strongly connected through the brain via various pathways called the gut-brain axis, and these connections indicate that the state of the gut has an influence on the state of the brain and behavior. There are various ways in which gut-brain axis therapies can improve in ASD patients, including manipulation of the microbiome via pro – and prebiotics, strict dietary options and through delivery of a fecal microbiota transplant (FMT). These interventions will be further discussed a later section. First, it is important to elaborate on the concept of the gut-brain axis.

The gut-brain axis

There is a strong, bidirectional connection between the gut and the brain, called the gut-brain axis (GBA). Via these connective mechanisms, brain signals have an influence on gut physiology and the composition of the gut microbiome, while intestinal signals and gut microbiota can influence the brain physiology the other way around. Pathophysiology of this connection is known to have effects in a wide range of neuropsychiatric disorders like depression & anxiety, schizophrenia, and bipolar disorder, but also in neurodevelopmental disorders like ADHD and ASD [16]. There are a handful of pathways through which these signals are conveyed, which are schematically outlined in Figure 1 [17].

Neuroanatomic pathways

First, signals can be sent via the enteric nervous system (ENS, the nervous system of the gastrointestinal tract) and the autonomic nervous system (ANS, regulates autonomic functions like heart rate and gut contraction). These are signals from brain to gut and can be either sympathetic (often stress induced), which slows down digestion by decreasing peristaltic contraction and overall intestinal muscle tone, or parasympathetic (often in a state of rest), which upregulates digestion by increasing peristaltic contraction and overall intestinal muscle tone [16].

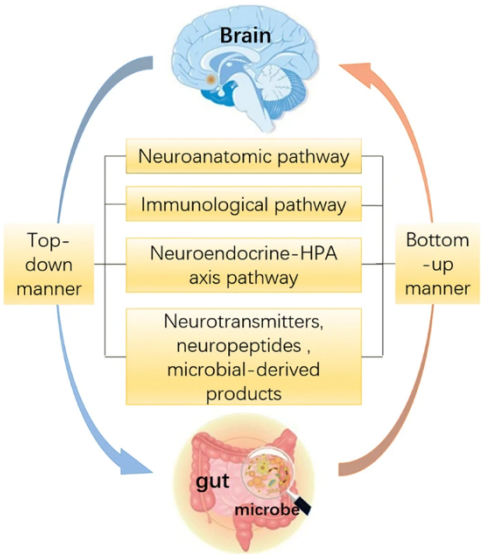

Figure 1: The gut-brain axis. Gut-brain bidirectional communication is conveyed through neuroanatomic, immunological, neuroendocrine, and microbial metabolite pathways.

Other nervous signals can be sent via the vagus nerve (the largest nerve of the ANS) from the GI tract to the brain stem, involving the hypothalamus (link between nervous system and hormonal system) and the limbic system (regulates emotions). Through these pathways, gut microbiotas have impact on brain physiology. These vagal afferent signals can be induced by inflammatory cytokines of the gut wall mucous membrane, that are possibly produced in reaction to the gut microbiota [16]. A study by Lyte et al. (2006) showed that a bacterial infection of *Citrobacter rodentium* in mice upregulated c-Fos protein levels in the afferent spinal nerves. C-Fos is known to be essential in cell division and proliferation, thereby promoting neuronal connections. The infection caused anxiety-like behavior in the mice, and it was found to be very likely due to the sensory vagal connections of the gut [18].

Neuroendocrine pathway

Next to nervous communication, a connection from brain to gut is also constituted trough the neuroendocrine (hormonal) system. The so-called hypothalamic-pituitary-adrenal (HPA) axis is the link between the brain and the body, with hormones as its messengers. The HPA-axis mediates emotions, immune functions, stress responses and mood disorders. The HPA-axis is responsible for the production of cortisol, the stress hormone, in the adrenal gland. Strong HPA-axis activation and subsequent high levels of cortisol have been found to result in enhanced gut motility and transformation of the gut microbiota composition [16].

Immunological pathway
Gut microbiotas also influence the mind by acting on the immune system. Intestinal (pathogenic) microbes excrete compounds that induce specific immune responses. These immunological activation in turn causes production of pro-inflammatory cytokines (signaling molecules of the immune system) which also enter the brain by crossing the blood-brain barrier (BBB), thereby modulating brain physiology. These increases in pro-inflammatory cytokines are related to neuropsychiatric disorders such as depression and anxiety [16].

Microbial metabolite pathway
Metabolites produced by the microbiotas have a signaling function as well. Two classes of metabolites are of most importance.
Short chain fatty acids (SCFAs) are produced by gut microbes in the process of dietary fiber fermentation in the intestines. SCFAs can modulate brain activity in several ways. They can act on enteroendocrine cells in the GI tract, which in turn produces neuropeptide compounds that can initiate nervous signals in the enteric nervous system to the brain. Second, SCFAs can reduce the production of pro-inflammatory cytokines and increase production of anti-inflammatory cytokines, which in turn can cross the BBB and enter the brain.
Monocarboxylate transporters have also been found to be extensive in areas around the brain. These types of transporters can transport SCFAs into the brain because of their carboxyl group. Lastly, SCFAs can directly act on fatty acid receptors in the spine and thereby activate the afferent neurons of the spinal cord and ultimately neurons in the brain.
Gut microbiota are also responsible for regulation of (monoamine) neurotransmitters. Approximately 95% of serotonin is produced in cells of the colon, and gut microbiota are thought to partly control this. For instance, it was found that fecal metabolites of indigenous bacteria directly stimulated serotonin production in cell cultures from the colon. Next to this, mice that were kept germ free (GF) were found to have lower levels of both dopamine and noradrenaline, suggesting that gut microbiota have a role in the synthesis of these neurotransmitters. Lastly, levels of the inhibitory neurotransmitter GABA - which can actively cross the BBB and influence brain physiology – were exceptionally lower in GF mice. Therefore, monoamine neurotransmitters and GABA are suggested to play an essential role in microbe-brain interactions [16].

Summarizing, gut-brain interactions are extensive and proceed through a variety of routes. Bidirectional communication is conducted through efferent connections between the enteric and autonomous nervous systems & afferent connections via vagal and spinal projections, neuroendocrine signals, neuroimmune signals and bacterial metabolites.

The gut-brain axis and ASD

It is clear to see that ASD often co-occurs with morbidities in the gastrointestinal (GI) tract. It has been reported that 9-84% of children with ASD have GI tract symptoms (depends on the method of the study) in comparison to 9-37% for non-ASD children. All relevant studies summarized revealed that ASD indeed causes a greater risk for GI symptoms. Interest in this correlation also arose through GF mice studies. These mice showed a higher level of social impairments compared to a control group, and this suggested that gut microbiota might have an important function in development of social behavior [19]. There are a few hypotheses regarding the comorbidity between ASD and GI issues [20]. Gene & environment interactions are most likely to be responsible in most of the etiologies [8]. There are various GI pathways that are altered in ASD.

Some metabolites involved in the functioning of the gut and microbiota are related to ASD. SCFAs are particularly interesting. SCFAs possess the ability to cross the gut-blood-barrier (GBB) and subsequently cross the BBB and enter the brain. Propionic acid (PPA), an SCFA, has been found to be elevated in the feces/urine samples of ASD-children. Elevated SCFA levels are known to be associated with GI symptoms [20] and PPA can induce ASD-like behavior in mice [21]. Free amino acids (FAAs), which are derived from protein degradation, also have higher levels in fecal samples of ASD children. Some of these FAAs, like glutamic acid, have been directly linked to ASD. Normalization of the gut microbiota and metabolite production could have positive effects on ASD behavior [20].

The immune system pathway of the gut-brain axis is affected in ASD as well. Individuals with ASD have increased levels of pro-inflammatory cytokines in their blood plasma. This can cause inflammation in the gut and combined with toxins from pathogenic bacteria can cause increased permeability of the gut. This allows the gut microbiota to get through the intestinal wall into the internal system and activate the mucous membrane. As earlier mentioned, these mucous cells will then produce its own inflammatory cytokines and activate the vagal system and subsequently modulate CNS (therefore brain) activity. Also, because of this impaired gut permeability, toxic bacterial metabolites like lipopolysaccharide (LPS, large bacterial membrane molecules) can enter the internal milieu and activate an immune response through Toll-like receptors. These receptors are specialized in recognizing bacterial compounds and result in a strong immune response in the ENS and CNS [20].

There is also an association between GI-mediated neurotransmitters and ASD. Blood serotonin is known as an ASD biomarker and is present in about 30% of ASD individuals. A mouse model that had hyperserotonemia, which means a higher blood serotonin level, showed ASD-like behavior: disrupted social behavior, abnormal communication, and repetitive behaviors [20].

Lastly, also maternal factors are observed both in animal and human studies to have an influence on ASD prevalence and GI functioning. Particularly, maternal obesity and diabetes are known to play a role; ASD is 1.5 more prevalent in children of these groups. A maternal high fat diet (MHFD) has been coupled to microbiota dysbiosis, both in children and the mother. In an animal study, a MHFD in mice resulted in pups that had a gut microbial dysbiosis, reduced social behavior, fewer oxytocin neurons and reduced synaptic plasticity in parts of the reward system [8]. In the offspring of MHFD mice, the colonization of the bacterium *L. Reuteri* was found to be impaired more than 9-fold. Treatment with *L. Reuteri* restored the social behavior, oxytocin levels and synaptic plasticity, strengthening the evidence that GI issues and ASD are connected [22].

Thus, there is a connection between the gut and ASD, and possible microbiota and dietary changes could normalize the afferent & efferent communication and modulation that contributes to ASD.

Treatment of the GI tract in ASD

To possibly alleviate symptoms associated to ASD, targeting the GI issues is deemed a new promising prospective. There are a few ways in which this can be achieved and can be combined to constitute a concise treatment program. The state of the gut relies on the state of the gut microbiota as earlier described, so manipulation of the gut microbes is an interesting target to investigate.

Fecal microbiota transplantation

The most effective method to change the composition of the gut microbiota is through a fecal microbiota transplantation (FMT). This transplantation involves the administration of feces of a healthy donor into the intestinal tract of a recipient with a deviant microbial composition. The effects have been studied for ASD treatment. In a trial with 18 patients with both ASD and concurring GI issues, treatment started with administration of the antibiotic vancomycin and a bowel cleanse in preparation of the FMT. An extended FMT with a high initial dosage was performed followed by daily oral maintenance doses for 7-8 weeks. After this process, short-term effects were tracked for 8 weeks. Compared to baseline behavior, the ASD symptoms and its severity were significantly improved. The participants were again checked 2 years post treatment and still, the symptoms continued to improve. Next to behavioral improvements, GI symptoms also reduced in the participants by an average of 58% both at the end of treatment and after the two-year follow-up [23, 24]. However, critical evaluation of this study revealed inconsistencies. There was no placebo group, and 12 out of 18 patients changed their medication, diet, or nutritional supplements during the study [25]. All in all, results look promising but further large-scale research is required to study its potential.

Pro- and prebiotics

Another possible intervention category is administration pro- and prebiotics. Probiotics are supplementary bacteria that help in constructing a healthy microbiome. Probiotics have been shown to have effects in rodent studies; as earlier mentioned, *L. reuteri* restored social behavior in MHFD mice offspring [22]. The current evidence for beneficial effects of these pro/prebiotics have been observed but are yet limited in human studies. For instance, a study by Parracho et. al (2010) perfomed a study on administration of the probiotic *Lactobacillus plantarum* to children between 4-16 years old. This resulted in a significant alteration of the composition of the microbiota, with increases in *enterococci* and *lactobacilli* groups and a reduction in *clostridium*. Generally, ASD patients are known to have lower levels of enterococci and higher levels of clostridium [26], so these results appear to be effective in restoring dysbiosis. Behavioral scores were improved compared to baseline and this suggested that supplementation of *L. plantarum* was effective [27]. However, the reporting of the results was unclear; the probiotics and placebo group were not compared in the statistical analysis and in the follow up results of the study, 45 out of 62 participants withdrew from the study. Another study by Liu, Wan et. al (2019) reported no difference in placebo and *L. plantarum* groups. However, improvements were noted in certain traits like anxiety and inattention, but only for the group of participants between 7-12 years, while those between 13-15 years did not improve. Another study by Santocchi et al. (2020) had similar results, except that ASD children with GI symptoms experienced improvements in the GI symptoms but also in adaptive ability and multisensory processing (seen as core ASD symptoms) compared with placebo [28]. All around, probiotics seem to be promising but because of the wide variety of probiotics and study methodology like dosages and duration, no general evidence can be concluded.

Prebiotics are non-digestible nutritional compounds that promote the growth of healthy microbes in the gut. Not many publications have been done on the effect of prebiotics in ASD, but effects have been noticed. Grimaldi et al. (2017) studied the effects of galactooligosaccharide (GOS) between children with ASD and controls. Significant increases in *Bifidobacteria* were noted in both groups. Next to this, SCFA production was altered, reducing PPA and increasing butyrate. As earlier mentioned, PPA has been linked to ASD. Again, there are potential benefits to administration of prebiotics, but more extensive research is necessary [29].

Dietary interventions
Dietary interventions are already widely used in ASD treatment. 15-38% of children with ASD are on a restrictive diet, with the most common one being a gluten and casein free diet. This diet was developed out of the hypothesis that people with ASD have issues to digest these proteins and absorb peptides related to gluten/casein in their gut walls. The found evidence for this diet have not been successfully replicated in double-blind placebo-controlled studies [30], and it therefore might be smart to replace it with other dietary options. A better option may exist in the category of dietary supplementations. A recent meta-analysis (2019) showed that supplementation of omega-3, vitamins and/or other supplementations was more effective than placebo groups in improving core ASD symptoms (impaired communication, impaired reciprocity, and restricted/repetitive behavioral patterns) and ASD associated symptoms like anxiety, affective behavior, impulsivity, hyperactivity, and irritability. These results point towards a small and nonspecific effect of supplements in ASD, but more research is necessary as well to further confirm these effects [31]. Nevertheless, since supplementation is an easy option implement in ASD therapy, it may be more effective and easier than restrictive diets.

All things considered, the aim to improve GI symptoms to tackle ASD symptoms is logical but still limited in its options. More research is useful to constitute effective GI therapies for ASD treatment. Since ASD is such a heterogenous disease, there is no one-size-fits-all option. However, for those individuals who also face GI issues, relieving symptoms in the gut and restoring balance in gut microbiota is hopeful in ASD therapy.

Discussion

ASD is a frequently occurring neurodevelopmental disorder, affecting about 2% of the human population. It goes hand in hand with a long list of difficulties that mostly affect understanding communication and relationships. Interests and behavior are repetitive and restricted which may possibly be due to small clusters of overconnectivity in the brain. ASD is associated with various comorbidities like seizures, sleep disorders, psychiatric disorders and finally, GI disorders.

The cause of ASD is an interplay between genetics and environment. It has been indicated that ASD has a strong genetic origin. Current estimates point towards about 50% heritability. ASD is extremely heterogenous and involves several types of risk genes, like synapse formation proteins, transcription regulation & chromatin-remodeling proteins, and copy number variants. Environmental risk factors include natural environment factors, social and familial factors, and nutritional factors.

Correct treatment options are sought-after to improve social and cognitive functioning. Both pharmacological and nonpharmacological interventions can help with this, but a new promising alternative can be found in the co-occurrence of GI disorders. Since the gut is strongly connected with the brain via the gut-brain axis (GBA), improving symptoms of GI issues may positively influence ASD behavior. *L. reuteri* treatment in rodent studies showed that this is hopeful by improvements of social behavior, oxytocin levels and synaptic plasticity.

Currently, therapies targeting the GI are underdeveloped for ASD treatment. Although enough evidence exists that GI symptoms correlate with ASD behavior, no direct causation

has been established. FMT has shown to be effective, but the studies involved had some inconsistencies. The same truth holds for pro- and prebiotics. *L. plantarum* treatment showed behavioral improvements in several studies, but the significant results only had small effects. Studies with GOS showed a reduction in SCFA production, but no behavioral improvements were noted. Dietary supplementations have small and nonspecific effects on ASD symptoms. Concluding, more large-scale research is required in this field to establish the right GI-tract targeting treatment.

Speculation

Even though effects have shown to be small, I personally think that implementing these therapies still is a good idea. There is a long way ahead to find out which pro- and prebiotics are most effective in treatment, but there is no harm in administration of them. Since administration of pro- and prebiotics combined with dietary supplements is such an uncomplicated way to help in ASD treatment, I do not see why It should not be performed. Even though the effects are small, supplementary treatment can still help in alleviating symptoms which makes life for children and adults with ASD easier, and that is what matters for these people. These treatments are not going to prevent ASD, but all the small benefits are still worth it. As for FMT, more research is necessary to deduce the right treatment. Ultimately, I can see a future in which FMT, pro/prebiotics and dietary interventions can be combined to support ASD treatment for those who are suffering from GI symptoms. Although, it will only support the current interventions and will not become the figurehead of ASD treatment. I think that this position will stay reserved for pharmacological options, like oxytocin & vasopressin, which have proven to show significant acute and prolonged improvement in core ASD symptoms.

Source list

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