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EXPLORING SYNBIOTIC TREATMENT IN ADHD, AND IMMUNE-MEDIATED PRENATAL RISKS FOR NEURODEVELOPMENTAL DISORDERS

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Exploring synbiotic treatment in ADHD, and immune-mediated prenatal risks for neurodevelopmental disorders

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By

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To everyone who live with, to them who walk beside, to all of you who tirelessly support patients with neurodevelopmental and psychiatric disorders. Especially to all of you who contributed with your time and effort, literally your blood, to this research.

Popular science summary of the thesis

The normal gastrointestinal tract inhabits a vast amount of bacteria, viruses and fungi, collectively called the microbiome. For the last decades it has been suggested that these bacteria have far reaching impacts on us and our health, with the ability to influence our mood and behavior. This is possible through several pathways, together called the gut-brain-axis. The communication between the gut and the brain, the "axis", is in part modulated through the immune system. Common disorders, like eczema, and gastrointestinal disorders, and neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) are linked to the immune system. In addition these disorders share genetic connections.

Earlier research has proposed that children whose mothers have an activated immune system during pregnancy have higher risks of mental health problems. For example, children whose mothers had certain autoimmune disorders (ADs) during pregnancy, have higher risk of developing ASD. Also it is proposed that people with ASD can obtain positive effects by consuming specific bacteria. ADHD and ASD are related, but less is known about the gut-brain axis in ADHD. Further we don't know if other maternal ADs increase the risk for offspring mental health and if this impact is beyond ASD. To explore this, we let children and adults with ADHD consume either a mixture of probiotic bacteria and dietary fibers called Synbiotic2000, or a placebo (with no effect) during nine weeks. Thereafter, we analyzed if there were any changes in ADHD or other related symptoms. Further we investigated if we could find any effect on immune metabolites in the blood. It turned out that the children with ADHD who received synbiotic treatment had fewer autistic traits; they engaged less in typical autistic repetitive behaviors and stereotypic movement, and that the adults with synbiotic treatment felt less overwhelmed by strong emotions compared to those who had the placebo treatment. We also observed that the treatment had certain effects on analytes that are involved in inflammation and in the gut-brain axis. In another study we compared children who were born to mothers with or without an AD, e.g. Crohn's disorder, during the pregnancy, to determine if and when they developed a neurodevelopmental or psychiatric disorder and compared the risk between these groups. We saw that children whose mother had an AD disorder had higher risks of a mental disorders later in life.

We suggest that (i) ADHD related symptoms in part might be impacted by a bacteria supplementation called Synbiotic2000 and (ii) that the risk of developing a mental disorder might be a little bit higher if your mother has had an AD during pregnancy. However, we need more studies to investigate this and it is important to remember that the possible risk

estimates from these statistical analyses are risks at a group level and says little about the risk at an individual level. For example, if the risk is 50% higher, it could still be a quite small real risk. Let us say that the incidence of ASD in the general population is 2%. A 50% increased risk means that the risk has increased from 2 out of 100 till 3 out of 100.

In summary, this thesis explores the gut-brain-axis through bacterial supplementation using Synbiotic2000, and further examines the effects of prenatal exposure to maternal autoimmune disorders, assessing the risk for offspring mental health. We propose that targeting ADHD-related symptoms with Synbiotic2000 supplementation could be beneficial. Additionally, we observe that there is an increased risk of neurodevelopmental and psychiatric disorders in offspring exposed to maternal autoimmune disorders (ADs), suggesting that these groups might benefit from enhanced surveillance. This underscores the necessity for more longitudinal studies to further investigate these complex interactions and the underlying mechanisms linking the microbiota, the immune system, and neurodevelopmental and psychiatric disorders.

Abstract

The etiologies of neurodevelopmental disorders (NDDs) and psychiatric disorders are considered to be multifactorial, with both genetic and environmental factors. NDDs are associated with early atypical brain development and a significant genetic origin. Nonetheless, the genes behind the heritability are still not completely identified, and environmental factors are not fully mapped. Attention deficit hyperactivity disorder (ADHD) and Autism spectrum disorders (ASD) belong to the NDD family. Over the past twenty years, a growing body of evidence indicates an interaction between the gut microbiota and the central nervous system affecting behavior and ASD-like pathophysiology. This interaction is suggested to be mediated in part through the immune system and the bacterial fermentation products, the short-chain fatty acids (SCFAs). In animal studies, prenatal exposure to inflammation or a disturbed maternal gut microbiome has caused the offspring to display changes in CNS and behavior. Epidemiological studies have suggested that prenatal exposure to certain maternal immune mediated disorders such as autoimmune disorders (ADs) increases the risk for offspring ASD. Immune activity upregulation has been shown in NDDs, primarily ASD, and some psychiatric disorders e.g. depression and there is an overrepresentation of co-occurrence of immune-mediated and/or autoimmune disorders (e.g., asthma and eczema) with ADHD specifically, and NDDs in general. Further, there is a high comorbidity between gastro-intestinal symptoms and NDDs, both ADHD and ASD in particular, which has been suggested to co-vary in symptom burden episodes. A few studies, mainly focusing on ASD, have suggested that treatment with oral probiotics can reduce core ASD symptoms. There is a high comorbidity between ASD and ADHD. However, the possible implication for adjuvant treatment through targeting the gut-brain axis has been investigated to a lesser extent in ADHD. In addition, a comprehensive range of prenatal AD exposures in relation to a wide spectrum of offspring NDDs or psychiatric disorders has been investigated to a lesser extent.

The aims of this thesis were to investigate (i) treatment effects with a synbiotic in ADHD on clinical and biological aspects, and (ii) effects on child psychopathology of prenatal exposure to maternal immune mediated disorders.

1. Investigate treatment effects of Synbiotic2000 on (i) ADHD symptoms, ASD symptoms, daily function and emotion regulation, N=248 participants included, 182 completers (study I) and (ii) plasma immune activity markers and SCFAs, N=156

participant completers with blood samples (study II) in children and adults through a randomized controlled trail (RCT) with a nine week intervention.

2. Investigate prenatal exposure to (i) maternal Crohn's disease (CD), N = 1 105 997 (study III) and (ii) a wide range of maternal ADs, N= 1 107 802 (study IV) on offspring NDDs and psychiatric diagnoses through registry cohort studies.

In the RCT there was a reduction in (i) ASD symptoms in children and (ii) emotion regulation difficulties in adults after intervention with Synbiotic2000 compared to placebo. The symptom improvement was most pronounced in the ADHD cohort with higher inflammatory, sVCAM-1, at baseline (study I). At baseline, adults with ADHD had lower levels of SCFAs and higher levels sICAM-1 and sVCAM-1 compared to controls. Children with ADHD had lower level of SCFAs, and higher levels of proinflammatory sICAM-1, sVCAM-1, IL-12/IL-23p40 and IL-2Rα, than adults with ADHD. In the child cohort, Synbiotic2000 significantly reduced IL-12/IL-23p40 and suggestively increased propionic acid. Synbiotic2000 suggestively decreased several additional proinflammatory markers in the child or adult cohorts (study II). In the epidemiological studies, children exposed to maternal CD prenatally had an increased risk of sleeping disorder, feeding disorder and incontinence compared to children without exposure. These associations were not explained by perinatal risk factors nor child inflammatory bowel disorder (IBD), (study III). In study IV, we assessed *in utero* exposure to several maternal ADs, and show that maternal AD overall was associated with a modestly increased risk for offspring mental disorders combined. Certain specific ADs contributed to a doubled risk for offspring diagnosis, these associations were (i) Systemic Involvement of Connective tissue (SIC) with sleeping disorder, (ii) autoimmune thyroiditis with ASD and (iii) pernicious anemia with other behavioral or emotional disorders (F98). Assessing several maternal ADs and a comprehensive range of offspring diagnoses enables comparisons between different exposures and outcomes.

In conclusion, this thesis demonstrates (i) a potentially significant role for Synbiotic2000 in managing symptoms closely associated with ADHD, (ii) higher baseline levels of certain immune activity markers in ADHD, and (iii) modest-to-moderate risk estimates for offspring NDDs and psychiatric disorders, typically manifesting in early childhood, after several prenatal AD exposures. Further studies are needed for confirmation of the findings.

List of scientific papers

I. **Skott, E**., Yang, L. L., Stiernborg, M., Söderström, Å., Rùegg, J., Schalling, M., Forsell, Y., Giacobini, M., & Lavebratt, C. (2020). **Effects of a synbiotic on symptoms, and daily functioning in attention deficit hyperactivity disorder – A double-blind randomized controlled trial.** *Brain, Behavior, and Immunity.*, *89*, 9–19. https://doi.org/10.1016/j.bbi.2020.05.056

II. Yang, L. L., Stiernborg, M., **Skott, E**., Xu, J., Wu, Y., Landberg, R., & Arefin, S. (2023). **Effects of a Synbiotic on Plasma Immune Activity Markers and Short-Chain Fatty Acids in Children and Adults with ADHD—A Randomized Controlled Trial.** *Nutrients.*, *15*(5). https://doi.org/10.3390/nu15051293

III. **Skott, E.**, Söderberg, G., Giacobini, MB., Chen, X., Lindqvist, D., Gissler, M., Sjöberg, K., Lavebratt, C. **Offspring exposed to Crohn's disease during pregnancy and association with psychiatric regulatory disturbances in childhood - a nationwide cohort study.** *Manuscript*.

IV. **Skott, E**., Nivins, S., Stiernborg, M., Fogdell-Hahn, A., Giacobini, MB., Gissler, M., Lavebratt, C. **Associations of prenatal exposure to maternal autoimmune disorders with a wide spectrum of psychiatric and neurodevelopmental disorders in offspring – a nationwide cohort study**. *Manuscript*.

Scientific papers not included in the thesis

- Yang, L. L., Stiernborg, M., **Skott, E.**, Söderström, Å., Giacobini, M., & Lavebratt, C. (2020). **Proinflammatory mediators and their associations with medication and comorbid traits in children and adults with ADHD**. *European Neuropsychopharmacology.*, *41*, 118–131. https://doi.org/10.1016/j.euroneuro.2020.10.005
- Yang, L. L., Stiernborg, M., **Skott, E.**, Gillberg, T., Landberg, R., Giacobini, M., & Lavebratt, C. (2022). **Lower plasma concentrations of short-chain fatty acids (SCFAs) in patients with ADHD**. *Journal of Psychiatric Research.*, *156*, 36–43. https://doi.org/10.1016/j.jpsychires.2022.09.042
- Stiernborg, M., Debelius, J., Yang, L. L., **Skott, E.**, Millischer, V., Giacobini, M., Melas, P. A., Boulund, F., & Lavebratt, C. (2023). **Bacterial gut microbiome differences in adults with ADHD and in children with ADHD on psychostimulant medication**. *Brain, Behavior, and Immunity.*, *110*, 310–321. https://doi.org/10.1016/j.bbi.2023.03.012

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List of abbreviations

AD	Autoimmune disorder
ADHD	Attention Deficit Hyperactivity Disorder
AID	Autoinflammatory disorder
AQ	Autism Spectrum Quotient
ASD	Autism Spectrum Disorder
ASRS	ADHD Self-Report Scale
BBB	Blood Brain Barrier
BMI	Body Mass Index
CD	Crohn's disease
CNS	Central Nervous System
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CVD	Cardio-Vascular Disease
DERS-16	Difficulties in Emotion Regulation Scale-16
DSM	Diagnostic and Statistical Manual of Mental Disorders
EFSA	European Food Safety Authority
FDR	False Discovery Rate
FFQ	food frequency questionnaire
GI	Gastro Intestinal
GPCR	G protein-coupled receptor
GRAS	Generally Recognized As Safe
GWAS	Genome Wide Association Study
HDAC	Histone deacetylases
HR	Hazard ratio

IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
ICD	International Classification of Disease
ID	Intellectual disability
IL	Interleukin
IQR	Interquartile range
IR	Inflammatory arthritis
JA	Juvenile arthritis
LC-MS	liquid chromatography–mass spectrometry
MBR	Medical Birth Registry
MS	Multiple sclerosis
MSD	Meso Scale Discovery
NDD	Neurodevelopmental disorder
PGN	Peptidoglycan
QPS	Qualified Presumption of Safety
RA	Rheumatoid arthritis
RCT	Randomized Controlled Trail
RRD	Register on Reimbursed Drugs
SAA	Serum amyloid A
SCFAs	Short Chain Fatty Acids
SCI	Systemic Chronic Inflammation
SCQ	Social Communication Questionnaire
SIC	Systemic Involvement of Connective tissue
sICAM-1	Soluble Intercellular Adhesion Molecule 1
SLE	Systemic Lupus Erythematosus

SNAP-IV	Swanson, Nolan and Pelham-IV scale
sVCAM-1	Soluble Vascular Cell Adhesion Molecule 1
T1DM	Type I diabetes mellitus
UC	Ulcerative colitis
WFIRS	Weiss Functional Impairment Rating Scale

Introduction

The interplay between neurodevelopmental disorders (NDDs), gut microbiota, the immune system and prenatal environmental factors presents a complex and intriguing area of study. Attention-deficit/hyperactivity disorder (ADHD), is a prevalent neurodevelopmental condition characterized by symptoms of inattention, hyperactivity, and impulsivity, ADHD has been extensively studied yet remains only partially understood. NDDs, including ADHD, are assocaited with early and atypical brain developemnt and psychatric disorders are commonly co—occuring. The etiology of NDDs is believed to be multifactorial, involving genetic predispositions and environmental influences, further inflammatory involvment and dysbiosis have been suggested. Among these influences, two are of focus in this thesis (i) the gut microbiota and (ii) prenatal exposure to maternal autoimmune disorders (ADs). Both aspects have emerged as areas of interest due to the potential impact on fetal brain development, subsequent behavioral outcomes in offspring, and the potential of risk reducing avenues and adjuvant treatment. The gut-brain axis serves as a communication pathway between the central nervous system (CNS) and the gastrointestinal (GI) tract, facilitated by neural, endocrine, and immune pathways. This interaction offers insight into how changes in gut microbiota may impact brain function and behavior. Probiotics, which are live microorganisms that confer health benefits to the host, have been suggested to influence the gut-brain axis through neural signaling, potentially affecting host behavior. Maternal autoimmune and autoinflammatory, conditions have been suggested to be associated with offspring ASD. Studies suggest that the immune dysregulation characteristic of these disorders could contribute to neurodevelopmental disturbances in offspring, potentially through inflammatory pathways that affect the developing fetal brain. Given the critical developmental windows during gestation, understanding if, which, and to what extent, maternal autoimmune conditions impact offspring development, is important. This thesis seeks to explore (i) levels of metabolites related to the gut-brain axis in ADHD, (ii) effects of treatment targeting the microbiome and (iii) prental exposure to a increased immune activity millieu, using two metodological designs. In a RCT (i) we analyse levels of SCFAs and immune mediating proteins in a ADHD cohort, and (ii) estimate treatment effects after treatment with a synbiotic during nine weeks, and (iii) through an epidemological design investigating if prenatal exposure to maternal ADs during pregnancy affects offspring risk for NDDs or psychatric disoders.

1 Literature review

1.1 Attention deficit hyperactivity disorder

Attention deficit hyperactivity disorder (ADHD) belongs to the neurodevelopmental disorder (NDD) family and is characterized by persistent patterns of inattention, impulsivity and hyperactivity with onset in childhood. In ADHD the core symptoms impair function across important life areas e.g. family, work, and school (1). Although ADHD is recognized as a disorder that often persists throughout the lifespan (2) there is a age depended symptoms decline (3). There are several subtypes of ADHD, with predominant inattentive symptoms, impulsivity/hyperactivity symptoms or the combination, however the treatment is generally the same (1). Emotion dysregulation, suggested to be a cardinal symptom, is a common associated feature in ADHD and has been associated with symptom severity and psychiatric comorbidities (4). Several theories concerning the etiology of ADHD have been proposed. Twin, family and adoption studies have shown a high heritability of approximately 70-80 percent, yet the molecular genetics is complex and genome-wide studies have only explained a part of this heritability (5-8). And even if genetics explains a portion, both environmental and social factors each play an important part (9) which may also explain the heterogeneity in symptoms and function (10). Assessment of environmental risk factors have methodological challenges, however reviews present some evidence for both prenatal and later in life exposure to e.g alcohol, maternal stress, deficiency in minerals or Omega-3 fatty acid, premature birth and low birth weight (11). The etiology of ADHD is indisputedly considered to be multifactorial (9).

The ADHD diagnosis is based on reported symptoms, but the body of pathophysiological evidence is growing (12). A review shows functional and structural changes in the central nervous system in ADHD (13), and several studies have shown delayed maturation of cortex in multiple areas and to some extent reduction in frontal cortex (12). Further delayed neuromaturation, synaptic pruning and myelination have been suggested (14). In addition, systematic reviews of neurobiological imaging have shown disruption in different regions in sub types of ADHD (15). It has been debated if the prevalence of ADHD is increasing (16) and potential overdiagnosis driven by milder or subclinical cases have been suggested (17). However, several meta-analyses suggest a stable prevalence during the last thirty years when controlling for methodology (18, 19). Recent reviews and meta-analyses asses the prevalence to ADHD in children to 7.6%, and 5.6% of teenagers (20). The prevalence of ADHD is higher in boys in childhood (21), the ratio between males and females becomes even higher

in adults (22). ADHD has been treated with central stimulants for 60 years (23), the medication is effective in reducing core symptoms of hyperactivity, impulsivity and inattention. The pharmacological treatment has less effect on emotional regulation. However the treatment is often discontinued (24) and in 7.4% of the cases the treatment is withdrawn due to adverse events (25). Long term effects of central stimulant treatment have been associated with increased risk of cardiovascular effects and a dose response relationship has been found (26), however this risk has not been shown in systematic review and meta-analyses (27). Treatment with stimulants has been suggested to significantly decrease the risk of negative functional outcomes (28), however the quality in some of the long term usage studies have significant limitations and the long term effects and risks are not clear (29). Treatment with central stimulants is suggested to be less well tolerated and less effective in comorbid cases (30).

1.2 Neurodevelopmental disorders

The NDD family, in addition to ADHD, consists of e.g. Autism spectrum disorder (ASD), Intellectual disabilities (ID) and deficits revolving around communication (i.e. stuttering) and learning (i.e. dyslexia) and are all connected to an early atypical brain development. The conceptualization of NDDs is originally based on the high comorbidity between the disorders and is in addition supported by shared genetics. ADHD and ASD have gained the most attention in the NDD group (31). ADHD and ASD in particular, have seven shared loci and five distinct ones, suggesting both a shared and a distinct genetic origin (32). It has been estimated that 33% of individuals with ASD have co-occurring ID (33). Comorbidity between ASD and ADHD is common and the lifetime prevalence of ADHD in ASD is around 40% (34), and the prevalence of ASD in ADHD is estimated to 21% (35). The shared/overlapping symptoms, the high incidence of both disorders in the same individual and the degree of shared genetics have raised the idea of NDDs as a continuum rather than a distinct diagnosis. In later years, both schizophrenia and bipolar disorder have been suggested to belong to the NDD continuum. However, both in the perspective of clinical diagnosis, treatment and research, the specific diagnosis is still treated as associated but yet separated entities (31). ADHD is characterized by difficulties regulation attention, activity and impulses (1). The closely related condition ASD is characterized by symptoms of impairment in social communication and repetitive behavior, presenting in early childhood (Lord et al., 2020). As for ADHD, there is no isolated cause for NDDs, the etiology is also regarded to be multifactorial with both genetic factors and environmental contributors (36-39). The prevalence of ASD is approximately 1% (33) and in contrast to ADHD there is no specific

treatment targeting ASD symptoms, however probiotics have been suggested as a possible treatment in ASD and have gained a vast interest. Two recent reviews, largely covering the same studies are inconclusive due to small sample sizes, different bacterial compounds being used and other methodological differences (40, 41).

1.3 Systemic inflammation

Systemic chronic inflammation (SCI) is implicated in the pathogenesis of various diseases, such as cardiovascular, autoimmune disorders (ADs), cancer, depression and neurodegenerative disorders, and has been suggested in NDDs. Systemic inflammation is characterized by persistent, non-resolving inflammation that escalates with age. SCI differs markedly from the transient, infection-induced acute inflammatory response. Evidence suggests that risks for SCI development could be transferred to offspring through epigenetic modifications shaping health cross generations (42). Dysbiosis have been associated with a number of autoimmune conditions (43) and psychiatric diseases (44). Dysbiosis is in turn associated with inflammation (45). Low grade inflammation in psychiatric disorders has been estimated to occur in approximately 20-40% of cases, and cytokine levels have been linked to symptom severity and in addition treatment response (46). Several studies have shown support for inflammatory involvement and upregulated cytokines (chemokines, interferons, interleukins, lymphokines, tumor necrosis factor) and soluble cytokines receptors in several psychiatric disorders (47-52).

1.4 Autoimmune disorders

Autoinflammatory and autoimmune conditions are part of a complex set of diseases marked by dysregulation of the immune system. Autoinflammatory diseases (AIDs), are more rare, and involve the innate immune system, while ADs, are more common and engage the adaptive immune system. Despite these differences, many conditions display characteristics of both AIDs and ADs. Researchers increasingly recognize that AID and ADs may not be entirely distinct, instead a continuum of inflammatory disorders is proposed and from now on referred to as ADs. Examples of diseases that exhibit features of both types of immune responses include e.g. Crohn’s disease (53). ADs consist of a broad range of diseases characterized by involvement of the immune system mistakenly attacking the body's own tissues, leading to a wide array of clinical manifestations. Fundamentally, these disorders stem from a loss of immunological tolerance, where the body's defense system fails to recognize self from non-self, initiating inappropriate inflammatory responses against its own cells and tissues. ADs arise from complex interactions between genetic predispositions and

environmental triggers (such as infections, chemicals, or dietary components), leading to the activation of autoreactive T and B cells. These immune cells produce inflammatory cytokines and autoantibodies, resulting in chronic inflammation and tissue damage (54). Inflammatory Bowel Disease (IBD), encompassing Crohn's Disease (CD) and Ulcerative Colitis (UC), are examples of immune-mediated conditions characterized by chronic inflammation of the GI tract (55), associated with disruptions in the gut microbiome and barrier function (56). ADs are broadly categorized into organ-specific disorders, where the immune response is directed towards a single organ (e.g., Type 1 Diabetes affecting the pancreas, Thyroiditis affecting the thyroid), and systemic autoimmune diseases, where multiple organ systems are affected (e.g., Systemic Lupus Erythematosus, Rheumatoid Arthritis). ADs are estimated to affect 5-8% of the population, with a higher prevalence among women. The chronic nature of these diseases, along with their ability to affect multiple body systems, makes them a significant cause of morbidity and challenges in healthcare management (57).

1.5 Symptom and disorder co-occurrence

In addition to the notable overlap of NDDs such as between ADHD, ID and ASD with co-occurrence estimated to approximately 20-40% (33-35), it has been estimated that over 50 percent of patients with e.g. ADHD also have at least one more psychiatric disorder (58). Recent reviews also support a high prevalence of mental health conditions in ADHD such as tic disorders, mood disorders, anxiety disorders, learning disabilities, sleeping difficulties (59, 60). In addition there is a significant co-occurrence between NDDs, psychiatric disorders and ADs. This relationship is bidirectional; chronic inflammation seen in ADs is suggested to contribute to the development of psychiatric symptoms, while stress and poor mental health can exacerbate autoimmune responses. Studies suggest that cytokines involved in autoimmune inflammation may also influence neurotransmitter systems related to mood and cognition. ADs affecting the GI tract, e.g. IBD, and CD have been associated with a high co-occurrence with psychiatric disorders (61-65). In CD the psychiatric symptoms are more pronounced in active disease periods compared to the milder ones (66). In addition there is a high co-occurrence of gastrointestinal (GI) symptoms in ASD (67) and ADHD (68-70). Studies have shown that comorbid GI symptoms are correlated with severity of psychiatric symptoms (71). However, a recent twin-study did not show an increase in GI symptoms in ADHD patients (72).

1.6 Gut-brain axis

The importance of the GI tract on human health has been a much discussed topic in the medical field for several decades. As early as in the 19th century the stomach was described as a second brain (73). In the 1980´s we begun to understand that the communication between the enteric nervous system and the central nervous system (CNS) was not mere a one way road (74). In fact, it seems to be a crosstalk often referred to as the gut-brain axis. This two-way communication, is in part modulated by the gut microbiota (75). There are several suggested communication routes, i.e. neural (e.g. vagus nerve), immune (e.g. cytokines), humoral and endocrine (e.g. the hypothalamic-pituitary-adrenal axis). The gut microbiota is crucial for the immune system homeostasis and dysbiosis can cause immune dysregulation, although dysbiosis is poorly defined. Commonly used definitions of dysbiosis are loss of diversity, loss of key taxa or higher abundance of pathogens (76). Dysbiosis is often associated with increased intestinal permeability and immune activation affecting the blood brain barrier (BBB) (77). The gut-brain axis is partially mediated by bacterial products found in the circulating system and with the ability to pass the BBB, e.g. short chain fatty acids (SCFAs) (78), Peptidoglycan (PGN) (79) and Hippurate (80). Microbiota derived metabolites have been demonstrated to impact brain development from early life stages (81). The composition of the gut bacterial community is influenced by several factors such as delivery route, bottle/breastfeeding, diet, and medication such as antibiotics. The colonization of the GI tract influences brain development and subsequently behavior and is unstable in the beginning of life (82), reaching a more stable state at approximately three years of. This period coincides with a critical time of brain development and synaptic pruning crucial for brain function (83). The microbial community has been shown to regulate microglia maturation (84) and further to be involved in maintaining homeostasis, barrier function and modulation of the immune system, important in nutrient digestion and in the protection against pathogens (85).

1.6.1 Microbiota, antibiotics, pre-, pros- and synbiotics

Microbiota, or the microbiome, is defined as the full community of bacteria, fungi and viruses existing in an organism. In this scope, microbiota refers to this complex ecosystem in the GI tract, but to be noted is not limited to this body site (86). Treatment with antibiotics do not only to target pathogenic bacteria but also beneficial ones. This can lead to imbalance in the gut microbiome (87) and exposure to antibiotics has been suggested to increase the risk of developing mental illnesses (88). Several animal studies have shown alterations in gut flora, brain development and increased autistic-like behavior in germ-free mice treated with high

doses of broad-spectrum antibiotics (89). In a register study, antibiotic exposure prenatally, gave a minor increase in the incidence of autism later in life (HR 1.11 CI; 1.03 - 1.19) with a higher risk with longer antibiotic exposure (90). Probiotics, prebiotics and synbiotics are designed to maintain or restore gut health (91). Probiotics are defined as selected strains of living organisms with beneficial effects to the host. Prebiotics are defined as a nonviable components, usually dietary fibers, with beneficial effects to the host through supporting beneficial microbiota. Lastly, synbiotics are the combination of pre- and probiotics (92).

1.6.2 Immune activity mediators

Immune mediators are crucial components of the immune system, helping to regulate the activity of immune cells, influence the inflammation process, and mediate the defense against pathogens. They include a diverse group of molecules such as cytokines, chemokines, and various other signaling molecules e.g. adhesion molecules. Cytokines are small proteins that are crucial in cell signaling. They can be pro-inflammatory or anti-inflammatory, however their effects are complex and the anti- or pro- inflammatory properties can be context dependent, helping to regulate the immune response. Chemokines are a subgroup of cytokines that specifically induce the chemotaxis (movement) of nearby cells, primarily leukocytes, guiding them to sites of infection or inflammation. There are Acute Phase Proteins, whose plasma concentrations increase (positive acute phase proteins) or decrease (negative acute phase proteins) in response to inflammation. This response is called the acute phase reaction e.g. C-reactive protein (CRP) which binds to the surface of dead or dying cells (and some types of bacteria) to activate the complement system and Serum Amyloid A (SAA) that influences the extracellular matrix and acts as an opsonin, are recruiting immune cells to sites of infection. Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin super family that facilitates leucocyte migration through endothelial wall as well as being connected to BBB function. The soluble form of ICAM-1, sICAM-1, is measurable in serum and CSF (93). In summary, these immune mediators are essential for the coordination and execution of effective immune responses, from the initial detection of a pathogen to the resolution of infection and the repair of damaged tissue. Their balance and regulation are critical; too little response can lead to uncontrolled infection, while too much can lead to chronic inflammation or autoimmune diseases (94, 95).

1.6.3 Short chain fatty acids

Short-chain fatty acids (SCFAs) are fatty acids with fewer than six carbon atoms, produced primarily through the fermentation of dietary fibers by the anaerobic microbiota in the colon. The most abundant SCFAs in the human gut are acetate, propionate, and butyrate. These metabolites play crucial roles in maintaining gut health and exert systemic effects on human physiology (96). Beyond the gut, SCFAs influence metabolic and immune processes. SCFAs have two main signaling pathways: inhibiting histone deacetylase (HDAC) and activating G protein-coupled receptors (GPCRs). HDACs are involved in controlling how genes are expressed, so when SCFAs inhibit HDACs, it can impact the development of various diseases, such as metabolic disorders, immune conditions, and cancer. Through GPCRs, SCFAs modulate the release of gut hormones, reduce inflammation, regulate blood pressure, and influence lipid metabolism. This has implications for e.g. metabolic syndromes where SCFAs can improve glucose homeostasis and increase insulin sensitivity. Further, SCFAs contribute to the regulation of the immune system. They enhance the production of regulatory T cells and the secretion of anti-inflammatory cytokines, playing a role in protecting against ADs (97). In summary, SCFAs are fundamental to gut health and exert wide-ranging effects on the human body, affecting metabolic, immune, and inflammatory pathways. Their production through dietary fiber fermentation underscores the importance of a fiber-rich diet for human health (96, 97).

1.6.4 Preclinical studies of the gut-brain axis

Studies conducted in germ-free mice and mice treated with antibiotics before birth have demonstrated that changes in the normal gut microbiota during early development can profoundly impact immune function, stress response, and behavior. These alterations have been linked to behaviors such as hyperactivity, depression, anxiety, ASD, and obsessive-compulsive behavior (98-101). Bacteria derived metabolites, associated with behavior changes in both animal and humans studies, have the capacity to cross the BBB and have been demonstrated to be present in animal models from as early as the neonatal period in the brains and suggested to influence neurodevelopmental outcomes that persist from the neonatal stage into adulthood (81). Rodents who received fecal transplantation from depressed humans expressed depression-like symptoms and changes in metabolism of tryptophan, a substance that is a precursor in serotonin and melatonin synthesis, and thereby have the ability to impact mood and sleep (102). In addition similar findings of symptom related behavior changes after fecal transplantation from patients into rodents have been shown in schizophrenia (103) and in autism (104). Rodent who received fecal transplants

from ADHD patients, compared to controls, reveled impaired connective integrity and changes in grey matter (105). Meta reviews on rodent exposed to maternal immune activation (MIA) prenatally show pathological changes in the activity of neural transmitters which in turn have been correlated with behavioral changes. This is suggested as a prof-of-principle evidence that a prenatal immune activation has the ability to impact molecular targets, synapse formation/function and thereby induce an imbalance in neural communication in offspring (106). It is not possible to draw conclusions for humans based on rodent studies, however, the results are consistent between animal and human studies in that certain ASD, depressive and anxiety symptoms are ameliorated after treatment aiming to restore gut health (107).

1.7 Clinical studies of mental health and immune involvement and gut-brain interaction

An upregulated immune activity has been associated with psychiatric disorders but the results remain inconclusive (46-52, 108, 109). It is hypothesized that these cytokines can cross the BBB, potentially affecting fetal neurodevelopment. The support for increased risk for offspring ASD and or psychiatric disorders of an *in utero* immune activated milieu is inconclusive. Several conditions such as obesity, low socio economic status, diabetes, and depression have been associated with higher inflammatory levels, and meta-analysis showed increased risks for both ASD and ADHD in offspring exposed to these conditions prenatally (110). Commonly investigated analytes associated with inflammation are C-reactive protein (CRP) and liver-derived acute phase proteins (SAA) (111).

1.7.1 Gut-brain axis mediators and immune activity in NDD

Associations between ASD and an altered gut microbiome have been shown in several studies (112). Investigating the microbiome, our lab has shown associations between ADHD and (i) diet in adults, and (ii) delivery route, stool consistency and probiotic use in children. Adults with ADHD had a difference in overall bacterial composition (beta-diversity) compared to neurotypical controls adjusting for age, sex, diet, stool consistency and BMI. The bacterial strain *Bacteroides stercoris* CL09T03C01 was found in significantly lower amounts in children with ADHD who were taking psychostimulant medication. *B. stercoris* was further positively associated with lower levels of formic and propionic acid (113). Other studies investigating the microbiome in ADHD have suggested differences, although meta reviews suggested that the evidence is inconsistent (114) and that there is still is no bacterial taxon associated with ADHD. Several of the previous studies did not adjust for diet (115). The gut-brain axis is mediated partly by bacteria derived metabolites produced by gut

microbiota e.g. SCFAs have been suggested to mediate the crosstalk between the gut and the brain (78, 116). Several bacterial derived metabolites have been demonstrated altered in ADHD, e.g. hippurate, a bacteria associated product, has been demonstrated to be elevated (80) and SCFAs lower (117). This has been shown also in ASD (118-121). It has been suggested that there is an altered CNS gene expression in some NDDs that is associated with an impaired BBB (122). Patients with ADHD have been reported to have higher levels of inflammatory markers suggesting a low-grade immune activation, however, the evidence is somewhat inconsistent and the main focus has been only in children (123-126). Offspring of obese mothers are at an increased risk of developing emotional regulation difficulties and ADHD (127, 128). Nielsen et al. found increased risks for offspring ADHD in relation to the number of inflammation related maternal states (129). Postnatal differences in microbial composition among children who later developed NDDs, i.e. ADHD and ASD have been suggested (130). Risk of offspring ADHD diagnose and sleeping impairment later in life have been connected to exposure to maternal antibiotic use, and is in addition dose dependent which strengthens the likelihood of causality (131). In addition, although the findings are inconclusive, a systematic review showed cytokine gene polymorphism associations with ADHD which might impact induction or release of cytokines. These associations could influence time of symptom onset, and one study detected cytokines in the cerebrospinal fluid (CSF) of ADHD patients (132). Increased levels of sICAM-1 have been suggested to indicate a compromised integrity of the BBB and has been found to be involved in schizophrenia, bipolar-disorder and depression (133) and ADHD (134). Zonulin, a peptide involved in tight junction function in the GI tract, has been shown to be elevated in ADHD suggesting an impaired integrity of tight junctions causing increased permeability (135). Inflammatory biomarkers in ADHD show considerable heterogeneity, however, the results are more conclusive with regards to inflammation during early life and later ADHD diagnosis (39).

Supplement interventions in NDDs

Dietary habits influence the composition of bacteria in the gastrointestinal (GI) tract, and various diets have been demonstrated to either increase or decrease inflammation in the host. Dietary interventions have been proposed to be a possible way of targeting the microbiome to ameliorate ADHD symptoms (136). The main focus for these inquiries have been ASD, suggesting potential treatment effects by treatment with pre- or probiotics (137). The results of postnatal intervention with probiotics are inconclusive, treatment with probiotic (*Lactobacillus rhamnosus* GG (ATCC 53103)) during the first months of life was associated with lower prevalence of ADHD and ASD later in life (130). However, a study with a similar

design and similar active treatment did not find such a association (138). Notably, a recent review and meta-analysis of supplement interventions studies of RCTs did not find support for probiotic treatment effects in ASD, although the studies were few and the treatment compound differed between the studies (139). There have been more limited studies investigating the possible therapeutics in ADHD, however recently a systematic review and metanalysis, notably including a study in this thesis (study I), did not show any treatment effects on ADHD symptoms (140).

1.7.2 Prenatal exposure to ADs and the risk for NDDs

Epidemiological research has suggested a potential association between parental ADs and offspring mental health, often with a continued primary focus on NDDs and ASD in particular. The incidence of ADs has been on an upward trajectory, exhibiting a higher occurrence in females (57). ADs are associated with NDDs (141, 142). Some ADs have been associated with offspring mental health. Several registry cohorts have investigated the risk for offspring mental health and exposure to prenatal ADs (142-152). The majority have had ASD as the outcome of intrerest (143, 144, 147, 148, 150, 152). The main exposure of interest has been parental, most often maternal, RA (143-145), and in addition SLE (147, 148) and DM (146). In several studies exposure to the maternal side is more strongly associated with offspring NDD suggesting that the association is not in full explained by genetic factors (142, 143, 146, 151, 153). However, in several studies consideration to perinatal risk factors has not been made (142) (152). Further the temporality aspects is not always taken into consideration and when it is, the results are still inconclusive. E.g. one study investigated exposure to RA and outcome offspring ASD and found an association only with exposure to maternal RA before/during pregnancy and not when the diagnose was received after pregnancy. However the risk of offspring ASD was equivalent with the risk in the negative control group (144). Another systematic review and meta-analysis showed an association between maternal life time RA and ASD in children, however pre-pregnancy maternal RA was not associated with ASD in children (OR = 1.45, p = 0.19, four studies) (143). An additional study, considering both time-point of RA and considering several perinatal risk factors, found that parental lifetime RA, primarily maternal, was associated with offspring ADHD HR 1.31 (95% CI 1.06, 1.63), however when adjusting for maternal smoking the confidence interval was widened and there was no longer an association, HR 1.32 (95% CI 0.80, 2.20). In addition, adjusting for APGAR 5 minutes, the association disappeared HR 1.24 (0.74, 2.05) (145). The pathophysiological mechanisms

underpinning this correlation remain largely unknown, and the precise and differential risk attributed to individual ADs has yet to be comprehensively characterized.

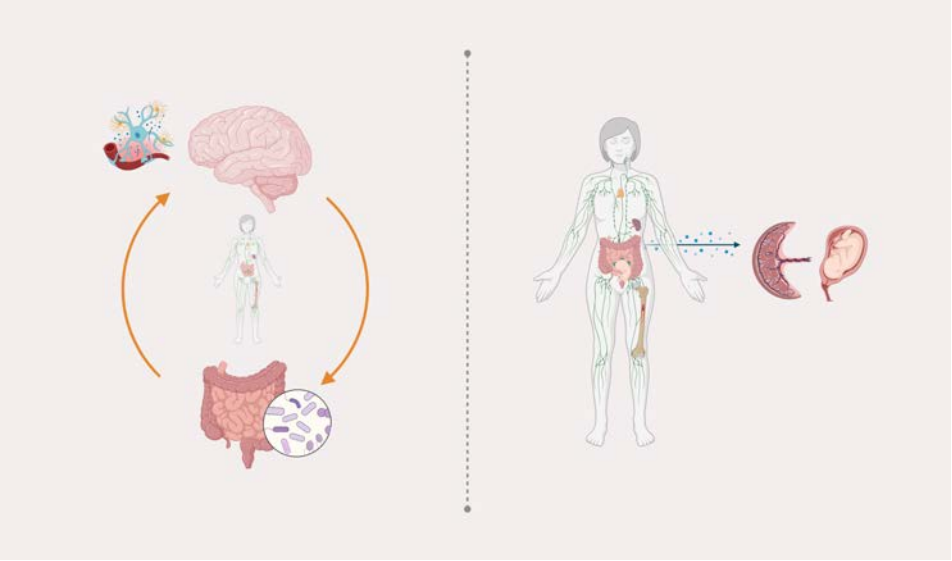

Figure 1. Simplified illustration of the proposed communication routes related to gut brain or prenatal exposure to immune mediated conditions. Created using biorender.

1.8 Summary

In summary, intervention studies investigating the potential of an adjuvant treatment targeting the gut-brain axis in ADHD is sparse, overall the sample sizes are small, the methodological differences are large, which obstructs the ability to draw conclusions from previous findings. Hence it is unknown if treatment with certain pre-, pro- or synbiotics, i.e. synbiotic2000, can be beneficial in ADHD. Prenatal exposure to maternal ADs have been suggested to involve increased risk for offspring adverse mental health, however often investigating a limited outcome i.e. ASD, or a specific parental AD or not considering possible mediators. A wider scope of exposure and or outcome would have the advantage of enabling comparisons between different AD exposures, and in addition, considering possible confounders and mediators would give a more robust design.

2 Research aims

This thesis aims to explore (i) new treatment avenues in ADHD through the gut-brain axis, (ii) associated analytes and (iii) asses risk of prenatal exposure to immune mediated conditions in relation to mental health, in two separate cohorts with different designs. We investigate treatment effects of a synbiotic in a ADHD cohort with a RCT aiming to investigate potential effects on (i) core and (ii) closely related symptoms and (iii) effects on biological markers associated with immune regulation and microbiota (study I and II). Further we aim to investigate if prenatal exposure to ADs impact risk for offspring mental health using a epidemiological approach. Using nation wide birth registries this thesis aim to investigate associations of prenatal exposure to (i) maternal CD and (ii) a wide range of maternal ADs with offspring mental health outcomes and consider both the temporality of the maternal diagnosis and possible mediadors such as premature birth (study III and IV).

3 Materials and methods

3.1 Cohorts

In this thesis two different cohorts were used, (i) the BAMBA cohort, a Swedish RCT was used in study I and II, and (ii) a Finnish birth registry cohort was used in study III and IV.

The BAMBA cohort and intervention study I and II

Cohort

Studies I and II are based on a cohort from Sweden called BAMBA, (ISRCTN57795429), with children and adults with ADHD, and neurotypical controls. The participants were recruited from January 2016 until June 2018. The cohort consisted primarily of patients from three psychiatric outpatient clinics in Stockholm, while some participants were recruited through advertisement in a local newspaper. Eligible criteria for participation were: ADHD diagnosis (DSM-V or ICD-10), age between 5-55 years, Swedish speaking/reading, and stable pharmacological treatment at a minimum of at least four weeks. Exclusion criteria were: ASD diagnosis, GI diagnosis (except IBS), celiac disease and diabetes, and lastly, antibiotic treatment the last six weeks. Recruitment and sample collection followed good clinical practice and was in accordance with the research protocol. All participants gave informed consent and were informed that they at any time could abort their participation. 248 participants were included at baseline. Participants were asked to contribute with symptom scales, food frequency intake; blood, fecal and urine sample (the two later not used in this thesis) at inclusion (baseline) and end of treatment (follow-up). The study was approved by the Regional Ethical Review Bord in Stockholm (Dnr: 2015/884-31, 2017/771-32, 2017/91-31, 2017/772-32, 2018/1132-32, 2018-1222-32).

Intervention

The participants were randomly allocated, via the Karolinska Trail Alliance, to one of the two treatment arms with allocation ratio 1:1 This computerized allocation was overridden in cases of several participants from the same family to reduce the risk of mix-up between intervention compounds. The allocations were unknown to participants and researchers until analyses were completed. Participants were instructed to (i) take the intervention once daily for nine weeks and to (ii) register intake on a paper and to return the empty compound bags to the research nurse when collecting new bags with a 1-2 week interval. The active treatment, Synbiotic2000, consisted of a lyophilized composition of (i) three lactic acid bacteria: *Pediococcus pentosaceus* 5-33:3/16:1 (strain deposit number: LMG P20608), *Lactobacillus*

casei ssp paracasei F19 (LMG P-17806) and *Lactobacillus plantarum* 2362 (LMG P-20606) ($3x10^{11}$ CFU per dose) and (ii) 10 grams of dietary fibers, equally proportions of beta glucan, pectin, inulin and resistant starch. Synbiotic2000 effects have been shown in previous research (154-160). The intervention with non-active treatment, the placebo, consisted of maltodextrin, a oligosaccharides with no known pre- or probiotic effects, and a record as being used as a placebo (161, 162). In total of the 248 participants who were allocated, 182 fulfilled the treatment and were included in the subsequent analysis. To be noted, not all provided blood samples (Study I and II).

Reference group in the BAMBA study

In study I and II neurotypical controls were recruited, N=61, in parallel with similar inclusion criteria as the participants in the BAMBA study but with no ADHD diagnosis The control group consisted of both those related to the participating patients (family member) and those unrelated. Information and biological samples were collected at one timepoint, and used in the case-control design (study I and II).

Finnish birth register cohort in Study III and IV

A database, of linked data from nationwide registers in Finland, containing all live births between (i) January 1st 1996 until December 31st 2014 followed-up until the 31st of December 2018 (study III) and 2021 (study IV) was used. The data were obtained from the Medical Birth Register (MBR), the Finnish Care Registers for Health Care (HILMO) and the Finnish Register on Reimbursed Drugs (RRD). The studies followed the recommended guidelines by STROBE Strengthening the Reporting of Observational Studies in Epidemiology. The cohort data were analyzed (i) between June 2022 and August 2023 (study III), and (ii) between October 2023 and February 2024 (study IV). MBR and HILMO are kept by the Finnish Institute for Health and Welfare, and RRD is kept by the Social Insurance Institution of Finland. The studies were approved by the Ethical review bords in Finland and Sweden (THL/1662/5.0500/2015, THL/1853/5.00/2016, THL/1496/5.0500/2019, 202303041-01, THL/5391/14.0200/2022).

Exposure study III

In study III the exposure was defined as maternal IBD, i.e. either UC or CD, and CD identified by ICD-10 codes K50 (CD) and K51 (UC) received before delivery. Additional exposures were maternal inpatient care during pregnancy as a result of the IBD (code O99.6) identified in the same databases.

Covariates study III

Three models adopted, (i) in model 1 adjustments were made for offspring birth year, sex, number of fetuses, maternal age at delivery, parity, socioeconomic status, marital status, mother's birth country, maternal smoking, psychiatric disorders, and use of psychotropic medication (ATC N05/N06), (ii) in model 2 addition adjustments in model 1 adjustments were made for maternal obesity (ICD-10 E65-66), pregestational diabetes (ICD-10 O24.1, E11, E14, ATC A10B), and systemic inflammatory disease (ICD-10 M30-36), and (iii) model 3 with adjustments as in model 1 and in addition child IBD diagnosis. Maternal psychiatric diagnoses were tracked using ICD-8, ICD-9, and ICD-10 codes from 1969 until delivery.

Exposure study IV

In study IV the exposure was maternal AD diagnosis in (i) the Connective tissue: RA (ICD-10: M05, M06; ICD-9: 714), Juvenile arthritis (JA) (ICD-10: M08, M45; ICD-9: 7143, 7200A), Systemic lupus erythematosus (ICD-10: M32; ICD-9: 7100A), other Systemic Involvement of Connective tissue (SIC) (ICD-10:M35.0, M35.1, M35.2, M35.3, M35.4, M35.5, M35.8, M35.9; ICD-9: 7102,7108,7112A,725, 729, 2794), (ii) the Endocrine system: T1DM (ICD-10: E10), Acute thyroiditis (ICD-10: E06.0, E06.1, E06.2, E06.5, E06.9; ICD-9: 2450, 2451, 2458, 2459, 2450A, 2451A, 2458X, 2459X), Autoimmune thyroiditis (ICD-10: E06.3; ICD-9: 2452A), Hyperthyroidism (ICD-10: E05.0, E05.1, E05.2; ICD-9: 2420A, 2420, 2421, 2421A, 2422, 2422A), (iii) the Digestive system: Celiac disease (ICD-10: K90; ICD-9: 5790A), Crohn's disease (CD) (ICD-10: K50; ICD-9: 5550, 5551, 5552, 5559), Ulcerative colitis (UC) (ICD-10: K51; ICD-9: 556), Primary sclerosing cholangitis (PSC) (ICD-10: K83.0; ICD-9: 5761X), Autoimmune hepatitis (ICD-10: K73.9, K75.4; ICD-9: 5714), (iv) the blood system: Pernicious anemia (ICD-10: D51; ICD-9: 281, 5351X), Systemic vasculitis (ICD-10: M30, M31; ICD-9: 4460A), (v) the nervous system: Multiple sclerosis (MS) (ICD 10: G35, G61.0; ICD-9: 340, 4149X, 3570A, 4100A) or (vi) the skin: Psoriasis vulgaris (ICD-10: L40, M07.0, M07.1, M07.2, M07.3; ICD-9: 696), Dermatomyositis/polymyositis (ICD-10: M33; ICD-9: 7103, 7104), Bullous skin disorders (ICD-10: L10, L12, L13; ICD-9: 694), Lupus erythematosus (ICD-10: L93; ICD-9: 6954A) received before delivery.

Covariates study IV

Covariates in the statistical analysis were: year of birth, sex, number of fetuses, parity/multiple births, maternal age, marital status, socioeconomic status, maternal birthplace outside Finland (yes/no), maternal smoking during pregnancy, maternal psychiatric disorder

and/or use of neurotropic medication (N05/N06) before pregnancy, maternal psychiatric disorder and/or use of neurotropic medication (N05/N06) during pregnancy,

3.2 Outcome measurements

3.2.1 Questionnaires in Study I and II

The participants filled in questionnaires on ADHD symptoms, ASD symptoms, daily functioning and emotional regulation pre- and post-treatment. Participants aged 5-17 were regarded as children and participants aged 18-55 as adults and were administered the corresponding questionaries. Children below 12 years got support from, or the questionnaires were filled in by, their caregiver. A retrospective food-frequency questionnaire (covering the previous 4 weeks), based on a previous studies ETICS (163), was used.

(1) ADHD symtoms were assessed with: Swanson, Nolan and Pelham-IV scale (SNAP-IV) for children and with ASRS (ADHD Self-Report Scale) for adults with nine items assessing inattention and nine assessing impulsivity and/or hyperactivity.
(2) ASD symptoms were assessed with: Social Communication Questionnaire (SCQ) for children and the Autism Spectrum Quotient (AQ) for adults. In SCQ the following domains are included: communication, reciprocal social interactions and restricted, repetitive behaviors and interests. AQ is subdivided into five domains: social skills, attention switching, attention to detail, communication and imagination.
(3) Function was assessed using Weiss Functional Impairment Rating Scale (WFIRS), divided into the six domains: family, work/school, lifeskills, self-concept, social activities and risky activities, different versions for child and adult.
(4) Emotional regulation was assessed in adults only using: Difficulties in Emotion Regulation Scale-16 (DERS-16), divided into five domains: clarity (lack of emotional clarity), goals (difficulties engaging in goal-directed behavior), impulse (impulse control difficulties), strategies (limited access to effective emotion regulation strategies) and nonacceptance (nonacceptance of emotional responses).

3.2.2 Blood measurements in Study I and II

Vascular inflammation marker measurements in Study I and II

Vascular inflammation, inflammatory cytokines, chemokines, and acute phase proteins in EDTA-plasma stored at -80C, were measured using the Meso Scale Discovery (MSD) platform. This method, based on sandwich immunoassay technology, enables the use of tailored multiplex assays for analyzing various molecules in a small sample volume. The procedure uses antibodies that attach to a specific spot in each well of the multiplex plate

which allows the target molecule from the sample to bind to the antibody-linker complex, which is then identified by another antibody tagged with an electrochemiluminescent marker, SULFO-TAG, facilitating the detection of the molecule. The intensity of the light emitted by the label, as measured by the MSD device, directly correlates with the concentration of the molecule present in the sample. In this study, a specific inflammatory profile consisting of 24 markers was evaluated using VPLEX (formally validated and have guaranteed performance specifications), as well as 3-spot, 7-spot, and 10-spot UPLEX assays, following the manufacturers' guidelines. Measurements were performed of 24 protein markers in the local laboratory by PhD Liu Yang; using the Panel 2 VPLEX Vascular Injury Panel 2 (human) Kit, for CRP, SAA, sICAM-1 and sVCAM-1, and U-PLEX Biomarker Group1 Human Multiplex Assays or the remaining (custom made design). However, due to analytes below detection level (over 25% below the minimum threshold), five markers were omitted. The observed median of both intra- and inter-assay variability (coefficients of variation) was maintained below 2.5% (ranging from 1.1% to 5.3%) (study I), and 9.9% (ranging from 4.6% to 16.9%), respectively (study II). All plasma samples were prepared using the same protocol and subjected to one freeze/thaw cycle prior to analysis.

Short chain fatty acids measurements in Study II

The nine SCFAs (formic, acetic, propionic, butyric, isobutyric, succinic, valeric, isolvaleric and caproic acid) were measured in EDTA-plasma and analyzed using liquid chromatography–mass spectrometry (LC-MS) at Chalmers University with minor modifications following a preset protocol (164). The samples underwent two rounds of measurement, spaced four months apart, with the first round utilizing six 96-well plates and the second round five. To mitigate round effects, twenty-two samples were tested in both rounds. Among the SCFAs, formic, acetic, propionic, succinic, and isovaleric acids demonstrated consistency across the two rounds, and thus, data from these acids were included in the final analysis. In each round the samples were assessed in batches. The median (range) intra-batch coefficient of variation (CV) was reported at 9% (ranging from 5% to 11%), based on two quality control samples analyzed on each plate. Inter-batch variation was adjusted for using these two quality controls. All plasma samples were prepared using the same protocol and subjected to two freeze/thaw cycles prior to analysis.

3.2.3 Outcome offspring ICD-10 F-diagnosis in study III and IV

Offspring were followed until time of event, being receiving an F-diagnosis identified through HILMO, or to end of follow-up being December 31st 2018 (study III) and December 31st 2021 (study IV).

3.3 Statistical analysis

Parametric T-test

T-test was used to determine if there was a significant difference between the means of continuous data before and after in e.g. the FFQ and were chosen upon that the data fulfilled the assumptions of normal distribution and equal variances (study I).

Non-parametric Mann-Whitney U test

Analyte level comparisons between groups e.g. ADHD participants/controls, were analyzed using Mann-Whitney U test, also known as the Wilcoxon rank-sum test, a non-parametric statistical test was used to compare differences between the two independent samples. It is particularly useful when the data do not meet the assumptions required for the t-test, e.g. normal distribution (study II).

Linear modelling

When evaluating the effectiveness of a treatment using a pre-posttest control group design, there are two main approaches to test for treatment effects. These are (i) ANOVA, analyzing the difference scores (subtracting pre-treatment scores from post-treatment scores), or (ii) ANCOVA, treating the post-treatment scores as the dependent variable while controlling for pre-treatment scores as a covariate (and possible also other covariates). ANCOVA was used in study I and II to analyze the data from the BAMBA RCT on the basis of its unbiasedness and better power in randomized studies (165). The levels of analytes in plasma were generally not normally distributed and were, therefore, natural log-transformed to meet the assumptions of parametric tests. We reported the 95% and/or 99% confidence interval (CI). Statistics was calculated with R-studio software (Study I and II).

Correlation

Correlation, is a statistical method used mainly to study the extent to which two quantitative variables are related. The most common is Pearson correlation test and is applied to assess the linear relationship between normally distributed variables. Spearman's rank correlation is particularly useful because it does not require linearity nor does it require that the variables are measured on interval or ratio scales (where precise differences between units are meaningful), making it more versatile in handling a wide range of data types e.g. psychiatric symptom scales to measures the strength and direction of association between two ranked variables. In addition, the use of rank rather than actual values makes it less sensitive for outliers. This method is widely applied in statistics when assessing the correlation between variables that are not necessarily normally distributed or when the data has ordinal

characteristics. The value of Spearman's rho ranges from -1 to 1. A high correlation coefficient indicates that the two variables have a strong relationship with each other. The relationship between marker levels as well as between marker levels and clinical symptom scores was assessed by Spearman's rank correlation. The coefficients (r) and p values were reported in the results (Study II).

Cox proportional hazard modelling

The Cox proportional hazards model is a semi-parametric survival analysis method frequently used in epidemiological research to examine the impact of various factors on the time to event (e.g., death or diagnosis). This model does not require specifying the underlying distribution of survival times, making it broadly applicable. It investigates the relationship between one or more predictive factors (e.g., socio-economic status or premature birth) and the occurrence of an event, taking time until the event into account, while also calculating the hazard rate, which indicates the rate at which the studied event occurs. Predictive factors defined as exposures can be either continuous or categorical. For categorical variables, one category is designated as the reference group. The hazard ratio (HR) then represents the comparison of the hazard rates between any given group and this reference group, where a hazard ratio greater than 1 indicates an increased risk, and less than 1 indicates a decreased risk, relative to the reference group. Assumptions should be fulfilled (i) the hazard ratio between groups is constant over time, and (ii) the relationship between the covariates and the hazard ratio is constant and not a function of time. Cox proportional hazard modelling was used in Study III and IV and the statistical analyses were performed with SAS.

Correction for multiple testing

This addresses the inherent risk of falsely rejecting the null hypothesis when conducting several tests on the same hypothesis. The Bonferroni correction is a widely applied method to adjust for this issue. It reduces the chance of false positives by dividing the threshold for significance (the alpha level) by the number of tests being conducted. Bonferroni correction was applied in Study II in the analysis on SCFAs. Although the Bonferroni method significantly lowers the risk of Type I errors (false positives), it is sometimes criticized for being too conservative and reducing the study's ability to detect real effects. Another approach is the False Discovery Rate (FDR). FDR controls the expected proportion of false discoveries among the rejected hypotheses, thereby offering a balance between identifying true effects and limiting false positives, this approach was applied in Study II in analysis of vascular inflammation markers. To be noted, in Study I no multiple test correction was

carried out. In Study III and IV, the focus was on effect sizes, but an attempt was made to adjust for multiple testing by using a 99% confidence interval.

3.4 Ethical considerations

All studies included in this thesis have ethical permissions, (2015/884-31, 2017/771-32, 2018/1132-32, 2018-1222-32, 2017/91-31, 2017/772-32, THL/1662/5.0500/2015, THL/1853/5.00/2016, THL/1496/5.0500/2019, 202303041-01, THL/5391/14.0200/2022). All participants in the RCT (study I and II) were informed that participation was voluntary, that they could retract their participation at any time without any impact to their standard care. Before inclusion all participants gave their written informed consent (young children consent were provided by the caregivers, adolescents by caregivers and the adolescent) and all data was handled in accordance with GDPR. All children were provided with a mild anesthetic patch before blood sampling, available also for adults. Children and adults who were reluctant to provide e.g. blood sample were invited to take part in the other aspects of the trial. The blood withdrawn were performed in accordance with clinical practice and entails a minor risk. The fecal and urine sampling were collected at home and entail no risks. The placebo treatment is commonly used in trails and safe, however contains, a small amount of carbohydrates why we as a precaution excluded participants with diabetes. The active treatment, Synbiotic2000, is a food supplement. The bacterial strains included have been granted Qualified Presumption of Safety (QPS) status as feed additives by the European Food Safety Authority (EFSA). Synbiotic2000 has previously been used extensively in research without any reported adverse effects, and is commercially available. The register cohort studies (study III and IV) did not require informed consent from the respective individuals as we had only pseudonymized data, and only data on group level were possible to export outside the Findata platform environment. Also, data from less than five in a group was not available. In addition, to consider risks, another ethical aspect of importance is potential benefits. If there is no, or very little, potential scientific gains to be made, the research is ethically difficult to justify. The knowledge gaps indicated in the thesis literature review, and potential clinical implications justify the low risks of our studies.

4 Results

4.1 Study I and II

There is growing evidence of an immune upregulation in several psychiatric and NDDs including ADHD (47-52, 123, 124). Modulating the gut flora have been suggested as a potential way to ameliorate inflammation and thereby psychiatric and NDD symptom burden (136), however there is a lack of RCTs in ADHD. We have previously shown, that certain psychiatric symptoms correlate with inflammation analytes in adults with ADHD, e.g. CRP and GI symptoms and sICAM-1 and ASD (166), which is in line with previous research (68-70). We hypotoseised that treatment with Synbiotic2000 would reduce core and related ADHD symptoms and further influence analytes associated with inflammation and related to the gut-brain axis. In the cohort there were N = 182 participants who completed the 9 week intervention and N=156 provided blood sample at basline and follow-up. In addition N=56 controls provided blood sample at one timepoint.

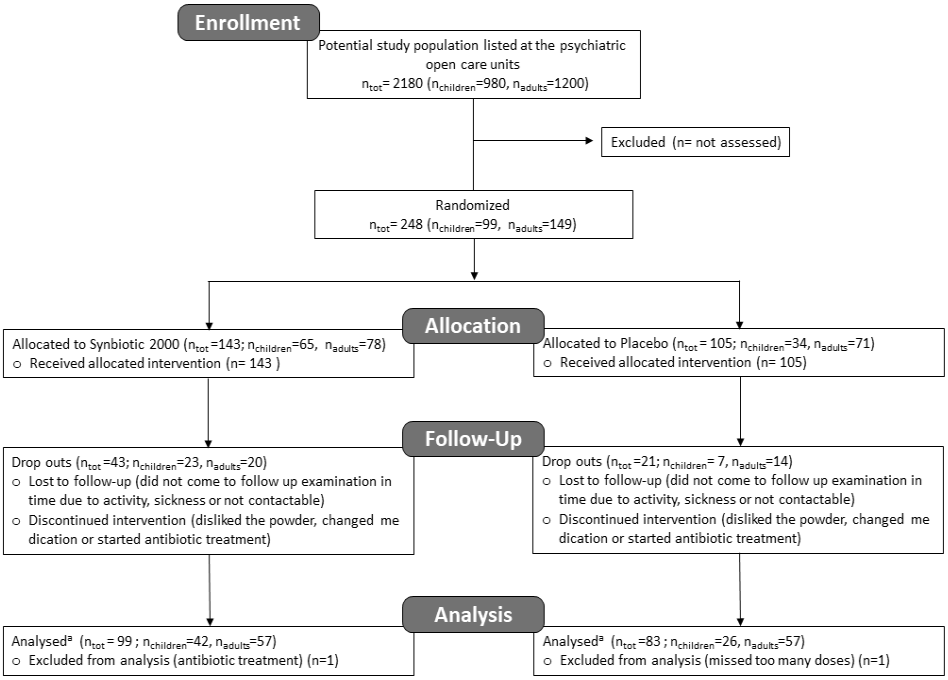

Fig 2. Flow chart of randomized controlled trail. Figure form Skott E*, Yang LL* et al., 2020, Brain, Behavior, and Immunity (167)

Results Study I

Primary endpoints

There was no significant treatment effect on symptoms when comparing synbiotic and placebo in neither of the primary endpoints, ADHD symptoms, functionality and ASD, with the exeption for a borderline synbiotic2000 specific effect in children on ASD symtoms (95% CI: −0.072, 0.003, $\eta^2 = 0.081$) and a significant improvement in ASD symptoms in the domain restricted, repetitive and stereotyped behaviors (95% CI: −0.166, −0.013, $\eta^2 = 0.052$; mean of t_1-t_2: 0.052 for Synbiotic 2000, −0.034 for placebo). In sensitivity analysis, stratifying on sVCAM-1 level, the effect of Synbiotic 2000 on ASD symptoms in children was significant in the high sVCAM-1 group, both on total scale (95% CI: −0.083, −0.001, η2 = 0.117) and on sub-scale restricted, repetetive and stereotyped behaviour (95% CI: −0.199, −0.006, $\eta^2 = 0.123$).

Secondary outcome

The secondary outcome was emotional regulatory difficulies in adults using DERS-16. Adults with Synbiotic2000 tretment improved in the sub scale of goal directed behaviour while emotionally upset (95% CI: −2.07, −0.014, $\eta^2 = 0.040$). In sentitivity anlays of the cohort with ADHD and above-median-sVCAM-1 at baseline there was a Synbiotic 2000-effect on the total scale (95% CI: −17.0, −2.40, $\eta^2 = 0.209$) and on four out of five subscales.

Results Study II

Plasma levels of immune activity proteins and short-chain fatty acids in ADHD at baseline

In the adult cohort with ADHD, at baseline, there were sigificantly higher levels of the proinflammatory cell adhesion molecules sICAM-1 and sVCAM-1, further the adult ADHD cohort had lower levels of the SCFAs formic, propionic, acetic and butyric acid, compared to controls. In the adult ADHD cohort, the analyte levels of sVCAM-1 and formic acid levels were correlated. In the child cohort with ADHD, there were to few child controls to perform case-control analysis. However, compared to adults with ADHD, the child cohort had higher levels ofsICAM-1 and sVCAM-1 and the cytokines, IL-12/IL-23p40, IL-2R, TGF-2B and TRAIL, and lower levels of eotaxin-1. Further, children with ADHD had lower levels of formic, acetic, propionic and butyric acid compared to adults with ADHD. In the cohort of children with current ADHD medication, the levels of sICAM-1 and sVCAM-1 were higher and the level of propionic acid lower, compared to the group of children with ADHD and no stimulant treatment. In the child cohort, sICAM-1, sVCAM-1 and eotaxin-1 where correlated

with acetic acid. To validate the detected negative correlations between SCFAs and immune markers, a preliminary cell experiment was conducted and showed lower sICAM-1 expression in cells preincubated with SCFAs.

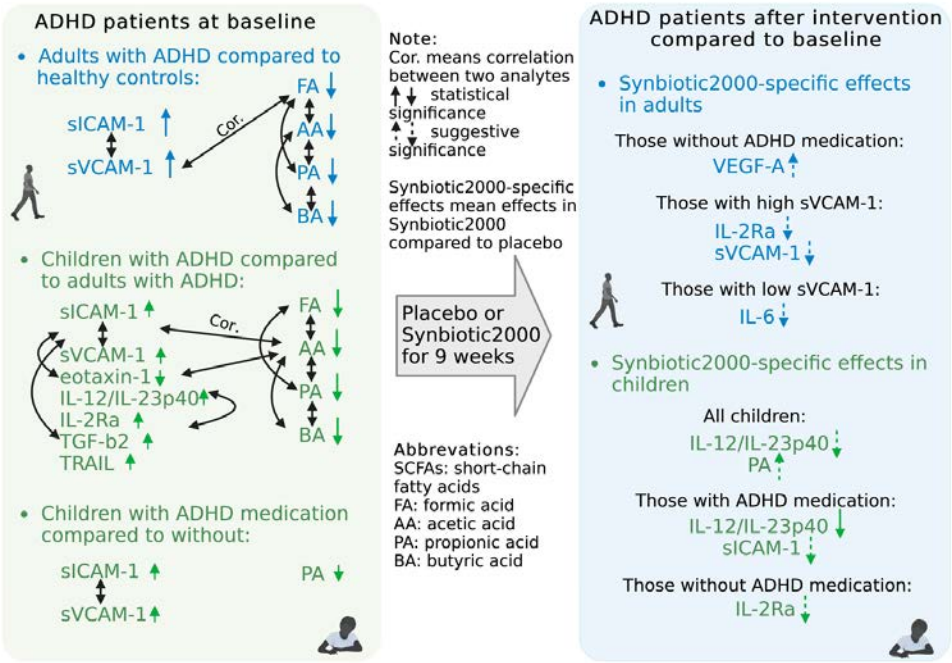

Figure 3 Summary of the findings. from Nutrients 6th of mars 2023. (168)

Effects of Synbiotic2000 on plasma levels of immune activity proteins and short-chain fatty acids in ADHD

At baseline there was no difference in analyte level between the two treatment arms: Synbiotic 2000 and placebo. After the nine-week Synbiotic intervention, there were suggestive Synbiotic-specific effects compared to placebo: (i) increased level of VEGF-A in adult group not taking stimulant medications, (ii) decrease in both IL-2Ra and sVCAM-1 in adults that initially had higher levels of sVCAM-1, (iii) reduction in IL-6 in adults with lower basleine levels of sVCAM-1. In children, there was a tendency of reduced IL-12/IL23p40 and increased propionic acid post treatment. In the child cohort with stimulant treatment there was a significant reduction of IL-12/IL23p40 and further a suggestive reduction of sICAM-1, and a suggestive reduction of IL-2Ra in children without stimulant treatment. The analysis were performed using ANCOVA.

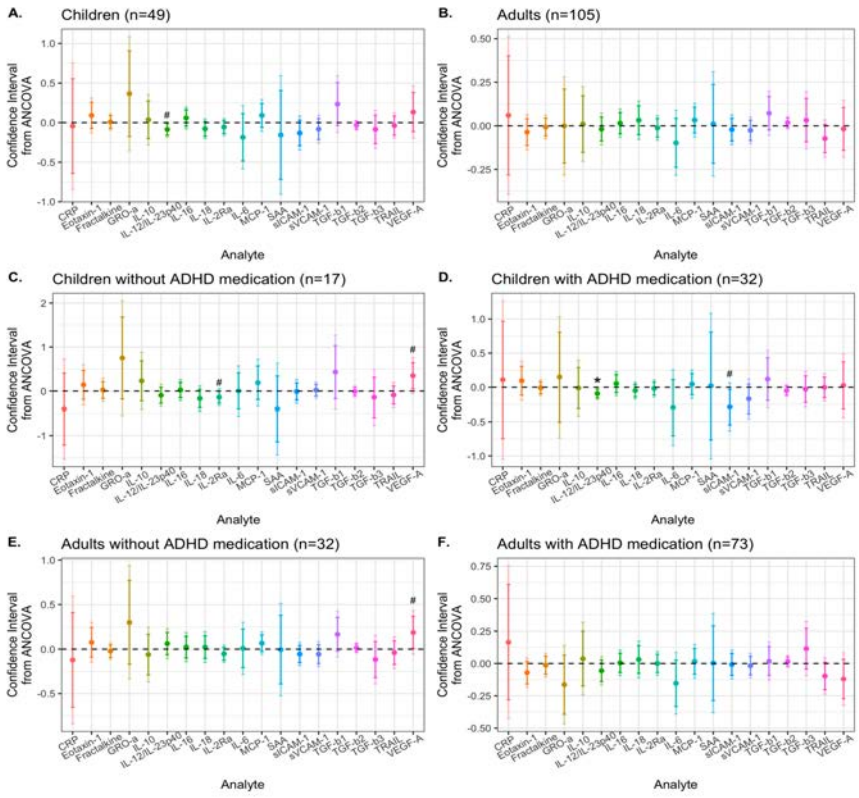

Figure 4. Confidence intervals (CIs) for treatment effects of Synbiotic 2000, compared to placebo and adjusted by sex, on immune activity marker levels. CIs were from analysis of covariance models for (A) children; (B) adults; (C) children on ADHD medication during the last 3 months; (D) children not on ADHD medication during the last 3 months; (E) adults on ADHD medication during the last 3 months; (F) adults not on ADHD medication during the last 3 months. Dark colors indicate 95% CIs, and light colors indicate 99% CIs. ADHD medication includes methylphenidate, dexamphetamine, atomoxetine, and for adults also lisdexamphetamine. A CI below 0 means that Synbiotic 2000, compared to placebo, reduced the analyte levels. * Statistical significance (α = 0.01); # difference at α = 0.05; Outliers (three for CRP and two for SAA) were excluded by the defined cut-off of more than 50* interquartile range (IQR) from the median. Figure from Nutrients 6th of mars 2023. (169)

4.1.1 Limitations Study I and II

In the RCT we investigated the impact of a synbiotic on psychiatric symptoms, and plasma immune activity proteins and SCFAs in a ADHD cohort. We found no Synbiotic2000 treatment specific effect on the primary endpoints including ADHD symptoms, however it is suggested that the treatment have possible effect in (i) reducing sub-clinical ASD symptoms in children and (ii) improve emotion regulation in adults, and further that improvements in this areas are more pronounced in the sub-cohort with higher plasma proinflammatory sVCAM-1 levels at baseline. Multiple testing correction was not done in Study I, increasing the risk for type I errors. However, in analysis of analytes multiple testing was adjusted for. From previous research we know that ADHD is associated with obesity and diet (170), we

therefore tried to control for diet and BMI, however we did not have BMI data in the child cohort. Recall bias might have been introduced in the use of a four week retrospective FFQ as participants might not accurately remember in detail what they have eaten, further the FFQ answers were in a second step converted to nutrient levels and amounts with a degree of approximation, possibly inducing measurement errors. Also, we lacked child controls, therefore possible associations in the child cohort are not necessarily specific to ADHD. Finally, in the placebo arm there was significant reductions in sVCAM-1 and sICAM-1 levels post treatment in the adult ADHD cohort which we could not explain.

4.2 Study III and IV

Studies suggest that maternal immune activation can impact neural transmitters and behavior in offspring (106) and prenatal exposure to maternal ADs has been suggested to increase the risk for NDDs in offspring (141-148, 150, 152, 171), however often not considering possible mediators or with e.g. a single. outcome of interest. We hypothesized that prenatal exposure to maternal ADs during pregnancy implies a higher risk for offspring mental health disorders later in life.

Results Study III

Our study on 13.8 million person-years revealed that offspring of mothers with CD have an increased risk of developing Incontinence, Feeding disorders and Sleeping disorders. Notably, exposure to severe episodes of CD during pregnancy, implied a 2-3-fold increase in the risk of Incontinence and Other feeding disorders in the children. These associations remained significant with a strict CI at 99%. Additionally, children exposed to maternal IBD also showed higher usage of hypnotics, antipsychotics, and sedatives. While factors such as maternal hospitalization could contribute to these findings, we ruled out such confounders. Furthermore, despite acknowledging that preterm birth and small birth size are risk factors for NDDs, it did not fully account for the observed increased risks in the offspring for the specific disorders mentioned.

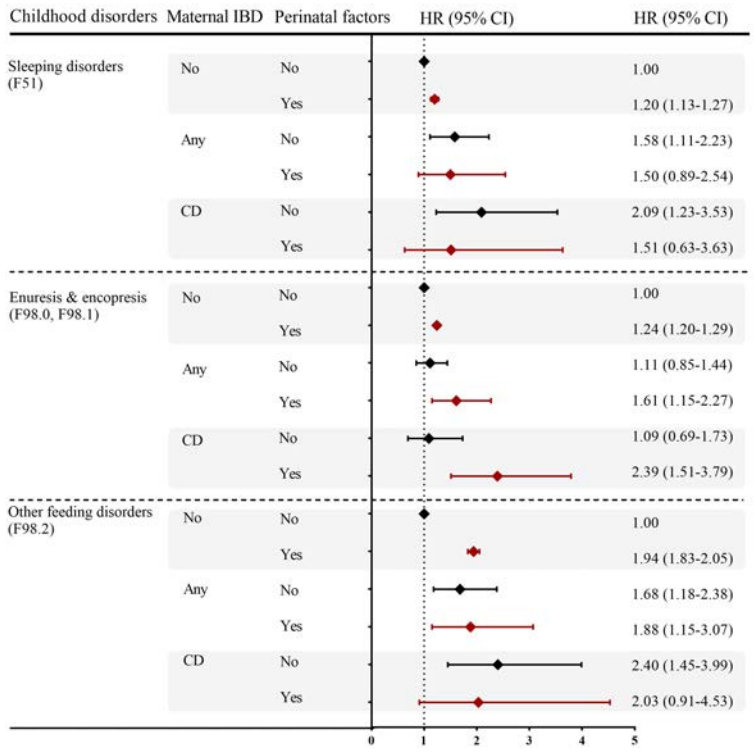

Figure 5. Psychiatric and NDD in offspring in relation to exposure to maternal IBD and CD stratified by perinatal risk factors. Forrest plot showing adjusted hazard Ratios (HR) for offspring psychiatric and mild NDD stratified on perinatal factors. Perinatal factors (yes/no) referred to birthweight below 2500 g, birth small for gestational age (SGA) and/or birth before the 37th gestational week. Reference group was offspring born to mothers without IBD. IBD: ICD-10: K50-K51; CD: ICD-10: K50. The analyses were adjusted for offspring birth year, biological sex, number of fetuses, maternal age group at delivery, parity, unmarried mother at birth, SES, mother's country of birth, maternal smoking, maternal in or outpatient psychiatric disorder and maternal use of psychotropic medication during pregnancy (N05/N06). Followed up until December 2018.

Results Study IV

Of the 1,107,802 births studied, 3.2% births were to mothers diagnosed with an AD pre-delivery. Offspring exposed prenatally to ADs had a 15% higher risk of major psychiatric disorders (HR 1.15, 99% CI 1.09-1.21) and an 18% increased risk of NDDs (HR 1.18, 99% CI 1.14-1.22). The risks were primarily moderate (less than doubled) e.g. HR between 70-99% higher risk for offspring ADHD (other disorders in systemic vasculitis), ID (type 1 diabetes), SDDs was found (acute thyroiditis and Crohn's disease), in three cases the risks were higher than two-fold i.e. sleeping disorders (M35), ASD (autoimmune thyroiditis) and other behavioral or emotional disorders (pernicious anemia). Offspring exposed to maternal ADs connected to the connective system and the endocrine system entailed the increased risks for several offspring F diagnosis. It was noted that the disorders with the highest effect sizes were neurodevelopmental, typically manifesting in early childhood.

Importantly, the study found that these associations were generally not influenced by adverse birth outcomes.

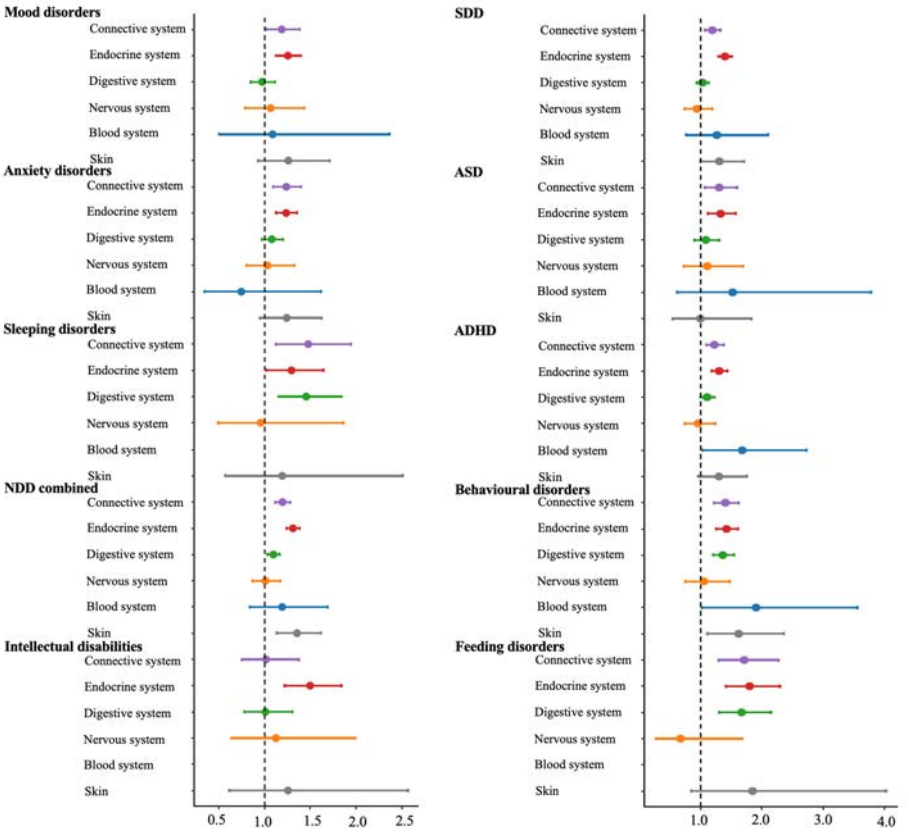

Figure 6. Associations between prenatal exposure to maternal ADs, grouped according to body system, and offspring psychiatric and NDDs (Hazard Ratios with 99% CI). Associations between prenatal exposure to maternal AD groups according to body system and offspring psychiatric and NDDs (Hazard Ratios with 99% CI). (i) Connective system (RA, JA, SLE, SIC), (ii) Endocrine system (DM1, Acute thyroiditis, Autoimmune thyroiditis, Hyperthyroidism), (iii) Digestive system (Celiac disease, CD, UC, Primary sclerosing cholangitis, Autoimmune hepatitis), (iv) Nervous system (MS, Guillain-Barre syndrome) (v) Blood system (Pernicious anemia, Systemic vasculitis), (vi) Skin system (Psoriasis vulgaris, Dermatomyositis/polymyositis, Bullous skin disorders, Lupus erythematosus) and offspring psychiatric and NDDs; Mood disorders (F30-34, 39), Anxiety disorders (F40-45, F48, F63.3), Sleeping disorder (F51), NDDs combined (F70-73, F79, F80-83, F84, F90-91, F93-95, F98), Specific developmental disorders (F80-F83), ASD (F84), ADHD (F90, F98.8), Behavioral and emotional disorders with onset usually occurring in childhood and adolescence (F90-F98) and Other feeding disorders of infancy and childhood (F98.2). Analyses were adjusted for: Year of birth, sex (boy/girl), multiple births (2 or ≥ 3), maternal age (≤ 24 or ≥35 years), first/only child (yes/no), marital status (married/cohabitating or unmarried/living alone), socioeconomic group (Lower white-collar, Blue-collar, Other, Upper white collar), migrant mother (yes/no), maternal smoking during pregnancy (yes/no), maternal psychiatric disorder (any F diagnosis, in or out patient visits) and/or neurotropic medication (N05/N06) before pregnancy (yes/no), maternal psychiatric disorder (any F diagnosis, in or out patient visits) and/or neurotropic medication (N05/N06) during pregnancy obtained from the MBR. Offspring were followed up until December 2021.

4.2.1 Limitations Study III and IV

In the context of prenatal exposure to ADs and its potential effects on offspring, both study III and study IV contribute valuable insights, albeit with inherent limitations typical of epidemiological designs. One critical limitation shared by both studies is the lack of genetic information, which could offer insights into the hereditary aspects of autoimmune diseases and their NDD impact. This gap highlights a common challenge in epidemiological research, where the inability to account for genetic predispositions can obscure the understanding of disease etiology and risk factors. Study III explored the association between the IBD diagnosis CD and various offspring disorders and found increased risks for offspring Sleeping disorders, Incontinence, and Other feeding disorders. Study IV provides a HR for a comprehensive exposures and outcomes. Both study III and Study IV have several limitations, notably the absence of data on breastfeeding, maternal medication beyond psychotropics, and paternal information. Additionally, we could not account for potential shared familial confounding factors. Another significant concern was the potential for sub-diagnostic AD pathology in children, raising the possibility that the observed associations could be partly due to undiagnosed AD-related pathology in the offspring. Further, despite taking into account the temporality of exposure and effect into consideration, both studies acknowledged the possibility that some mothers in the control group might develop ADs pre-pregnancy, but received a diagnosis post-partum, potentially confounding the results. In addition, both studies were also limited by methodological limitations common to epidemiological research, such as small exposure groups leading to low statistical power (Type II error) and the risk of sparse data bias (Type I error) due to multiple adjustments for potential confounders. Despite these challenges, the findings in study III are supported by several sensitivity analyses and study IV stands out for its novel approach in examining various maternal AD exposures and a range of offspring psychiatric and neurodevelopmental outcomes within the same cohort, offering a unique comparative perspective.

In summary, while both study III and study IV advance our understanding of the potential impacts of maternal ADs on offspring health, mechanistic and genetic research is warranted to further understand the interplay of immunological, inflammatory, genetic, environmental, and methodological factors that challenge epidemiological studies in this field.

5 Discussion

5.1 Introduction

The scientific exploration of the connections for NDDs and psychiatric disorders with the immune system and the gut microbiome is still an emerging field. This thesis aimed to explore (i) treatment with a synbiotic in ADHD and (ii) prenatal exposure to maternal ADs with outcome being offspring mental disorder, in a clinical and an epidemiological context, respectively. Studies I and II aimed to investigate potential effects of a synbiotic on psychiatric symptoms and plasma markers associated with the gut bacterial microbiome and immune regulation through an RCT design. Studies III and IV aimed to explore potential maternal ADs effects on offspring neurodevelopment with an epidemiological design.

5.2 Summary of findings across studies

In this thesis, we report levels of immune mediators and bacterial metabolites in ADHD, propose that synbiotic has the possiblity to ameliorate certain symptoms associated with ADHD, and that there are higher risks for NDDs and psychatric disorders in offspring exposed *in utero* to maternal ADs. In study I and II we measured both SCFAs and immune activity markers pre- and post synbiotic treatment and performed case-control comparisons and show that (i) participants with ADHD had higher levels of inflammatory markers, sICAM-1 and sVCAM-1, and lower levels of SCFAs, at baseline (study II), (ii) the cohort with higher baseline levels of these analytes was improved in common co-occuring psychiatric symptoms by the synbiotic treatment (study I), and (iii) there are synbiotic treatment effects on certain cytokines (study II). Study I and II suggest that, (i) there is an altered profile of immune activity and microbiome-associated analytes in ADHD compared to controls, (ii) children with ADHD have a more deviated profile of these markers than adults with ADHD, and (iii) after the intervention, targeting the microbiota, children and adults with ADHD are improved in symtoms related to ADHD, suggesting a gut-brain axis effect in-part mediated through immune system pathways. In study III we show that offspring, exposed to mothers with CD prenataly, had increased risks of developing incontinence, feeding and sleeping disorders later in life. This was supported by sensitivity analyses showing that (i) *in utero* exposure to severe CD episodes associated with markedly higher point risk estimates, and (ii) there was more dispensation of sedative/hypnotic prescriptions in the exposed group. In study IV, further investigating offspring risk for NDDs and psychiatric disorders, we show that (i) maternal ADs overall are associated with offspring NDDs and psychiatric disorders, and (ii) certain specific ADs such as and systemic vasculitis,

T1DM and acute thyroiditis are associated with NDDs like ADHD, ID, and SDD. The highest risks were observed for (i) sleeping disorders, (ii) ASD, and (iii) other behavioral or emotional disorders after exposure to maternal conditions e.g. autoimmune thyroiditis and pernicious anemia. Study III and IV, support the notion of a prenatal immune-mediated involvement in the etiology of NDDs.

5.3 The findings in the context of previous knowledge

Study I and II

Substantial evidence supports the existence of a gut-brain axis and its potential to influence neurological functions and behaviour in animal models (78, 98-106). The gut-brain axis is in part mediated by immune activity markers, and immune dysregulation is proposed in NDDs (118-121, 123-126) and ADHD (132, 134). Previous studies of immune activity markers in NDDs are often limited to a restricted number of analytes, for example a recent meta-analysis provide information on CRP, IL-1β, IL-6, IL-10, TNF-α and IFN-γ (126). Treatment with probiotics in ADHD has been suggested but the results remain inconclusive. A meta analysis from 2024, including 7 monoprobiotic or oligoprobiotics studies and one synbiotic study being our study I, of the therapeutic effect on ADHD symptoms report no effect on ADHD symtoms (140). However the included studies have large metodological differencies, (i) the use of different strains each with potentially diverse effects, (ii) the amount of bacteria used, (iii) singel or multiple strains and (iv) the length of the intervention, making inferences between the studies difficult. Further, there is a lack of biological data in these human interventional studies.

We assessed not only core ADHD symptoms, but we included ASD symptoms, functionality and emotion regulation. In addition to symptom scales we assessed immune markers which made it possible to stratify treatment effect analysis to those with higher immune activity at baseline, in line with a possible treatment effect mediated by an anti-inflammatory action. We provided levels for a wide range of immune activity markers and SCFAs in plasma with case control comparisons in the adult cohort, and show that adults with ADHD had higher baseline plasma levels of sICAM-1 and sVCAM-1 compared to controls. Previous research has linked sICAM-1 with psychiatric conditions and impaired BBB integrity (133). In addition children with ADHD had higher levels of sICAM-1 and sVCAM-1 compared to adults with ADHD, and furthermore, children with ADHD and current stimulant medication had higher levels compared to those without. We lack case-control comparisons in the child cohort, however increased levels of sICAM-1 have previously been reported in children with ADHD (134), which supports our findings. To be noted, sICAM-2 was lower in children on ADHD

medication (134) and sICAM-2 was not studied in this thesis. Notably, while both sICAM-1 and sICAM-2 are involved in mediating cell adhesion and immune responses, they do not necessarily correlate (93). In addition to elevated sICAM-1 and sVCAM-1 we found that the plasma levels of IL-12/IL23p40, IL-2Rα, TRAIL and TGF-β2 were higher, and eotaxin-1 lower in children with ADHD compared to adults with ADHD. We cannot conclude whether the child cohort baseline levels differ from a age matched control group however, IL-12/IL23p40 has also been suggested to be higher in ASD (172), schizophrenia (173) and in children with CD (174), where the two first disorders belong to, or are suggested NDDs, and the third often co-occurs with ADHD. Both ASD and CD were exclusion critera in the BAMBA cohort but we can not exclude the potential of prodromal symtoms or an undiagnosed medical condition. Further IL-2Rα and TGF-β2 have been associated with depression and schizophrenia (175, 176). In contrast to our results, with lower levels of eotaxin in children with ADHD compared to adults with ADHD, eotaxin has been showed to be increased in ASD, while the IL-12/IL23p40 closely related IL-12p70, which in accordance with our results was increased in ASD (177). Several immune signaling cells, including IL-2Rα, TRAIL, TGF-β2 and eotaxin-1 are associated with ADs (54).

We show lower baseline levels of SCFAs in adults with ADHD compared to controls and, lower SCFAs levels in children with ADHD compared to adults with ADHD. The lower SCFAs levels, is supported by a later study, showing lower levels of fecal SCFAs in an ADHD population and in addition showing a negative correlation between SCFAs and ADHD symptoms (117). We did not conduct a correlation analysis between plasma SCFA levels and ADHD symptoms. Most studies, assessing SCFA levels in humans use fecal concentrations, although the correlation with systemic SCFA levels in plasma is low (78) . The fecal SCFA level may have potential biases related to factors such as intestinal transit time, permeability, metabolite transport, and sample handling, and may not reflect physiological conditions in the colon (78). SCFAs are associated with dietary intake, particularly the consumption of fiber-rich foods, which affect their production by the gut microbiota. Our plasma SCFA levels were not significantly influenced by diet, but explained by number of antibiotic medication uses last 2 years and psychostimulant medication (178). In children there was a suggestive Synbiotic2000 increase of propionic acid. In ASD, SCFAs have been suggested to be both higher and lower, propionic acid-producing bacteria have been associated with symptom severity, and injection of very high levels of propionic acid in mouse brain is an established ASD-like model (78).

There were several suggestive treatment effects of Synbiotic2000, adults without stimulant treatment showed an increase in VEGF-A, adults with higher baseline levels of sVCAM-1 had suggestively reduced levels of IL-2Rα and sVCAM-1, and adults with lower levels at baseline showed a suggestive reduction of IL-6. A probable explanation to the detected IL-6 reduction is an increase in the placebo arm. VEGF-A is involved in angiogenesis (179) and has not been reported in ADHD previously, but has been associated with several ADs such as SLE, RA and MS (180). Both adults with higher sVCAM-1 at baseline and children without stimulant medication had a suggestive reduction of IL-2Rα post treatment, previously higher levels of IL-2Rα have been associated with disease severity in e.g. depression (169). In addition sVCAM-1 was suggestively reduced in adults in the cohort with higher inflammatory levels as baseline. Previously, meta-analysis of probiotic interventions in e.g. metabolic syndrome has shown treatment specific reductions of sVCAM-1 and sICAM-1 post intervention (181). In the child cohort there was a suggestive reduction of IL-12/IL-23p40, and in the cohort with stimulant treatment there was a significant reduction of IL-12/IL-23p40 and suggestive reduction of sICAM-1. Monoclonal antibodies targeting IL12-IL-23p40 is suggested as therapeutics in several ADs and are commonly used in IBD (174).

Further, we found synbiotic treatment effects on emotion regulation in the adult ADHD cohort with higher levels of inflammatory involvement at baseline. Previously, association between gut-microbiome beta-diversity and emotion regulation in healthy females was shown, which supports the gut-brain crosstalk hypothesis in our study, although also suggests that the finding might not be ADHD specific (182). However, there was a significant time-period between the stool sampling and the assessment of emotion regulation possibly biasing the study (183). In addition to the treatment effect in adults with higher levels of inflammation at baseline, children were improved in subclinical ASD symtoms, however there was no linear corrrelation for sICAM-1 or sVCAM-1 with symptom scale score for (i) DERS-16 in adult cohort or (ii) or ASD score for children in BAMBA (166).

Study III and IV

Investigating prenatal exposure to maternal CD, we show that children had higher risk of e.g. sleeping impairment supported by (i) higher point estimates with exposure to severe maternal CD and (ii) higher rates of related medication. Previous research has predominantly investigated ASD as outcome, with inconclusive results (151, 184-186). We simultaneously examined a wide range of maternal AD exposures and a comprehensive list of offspring outcome diagnoses of interest. Previous studies have had a more limited scope of exposures and/or outcomes of interest, and in addition often not considering perinatal risk factors (110,

143-146, 149-151). Investigating several exposures and outcomes in the same cohort enables the comparisons of effect sizes between different exposures. We found increased risk for overall mental health (HR 1.17; 99% CI 1.13-1.20) in exposure to any maternal AD which is in accordance with previous research (HR 1.16 95% CI 1.13-1.19) (151). The majority of the detected associations with childhood mental diagnoses were for the maternal AD groups affecting (i) the connective tissue, with the most prevalent diagnosis being RA and JA, and (ii) the endocrine system, with the most prevalent diagnosis being T1DM. We also found that offspring exposed *in utero* to systemic inflammatory conditions (SIC), such as Sjögren's syndrome, polymyalgia rheumatica, hypermobility syndrome, and other disorders of systemic involvement of connective tissue, had more than double the risk of developing sleeping disorders. While these associations have not been reported previously, it is possible that the increased risk observed may be related to these individuals experiencing a prodromal phase of ADs. Furthermore, exposure to autoimmune thyroiditis or acute thyroiditis was associated with a 1.5 to 2-fold increased risk for ASD. Maternal thyroid hormones, which are transferred to the fetus early in pregnancy (187), play a vital role in fetal brain development; deviations in their levels can influence this developmental process (187, 188). Also, exposure to pernicious anemia was linked to an almost threefold increase in the risk for behavioral and emotional disorders — a previously unreported association. Possibly, this stems from the condition's impact on Vitamin B12 absorption in the small intestine, due to autoimmune inflammation. Vitamin B12 is crucial for neurodevelopment, including neurotransmitter synthesis and myelination. Vitamin B12-deficiency during pregnancy is associated with delayed behavioral and cognitive development (189). Systemic vasculitis slightly increased the risk for ADHD and other behavioral or emotional disorders. Pregnancies complicated by systemic vasculitis are high-risk, as the condition can compromise blood vessels and placental function, affecting nutrient and growth factor delivery to the fetus (190).

Clinical relevance and implications

In study II, baseline assessments revealed that adults with ADHD had higher plasma levels of sICAM-1 and sVCAM-1 compared to controls. Notably, children with ADHD medicating with psychostimulants in the BAMBA study exhibited higher levels of sICAM-1 than those that were unmedicated. Given that psychostimulants are known to elevate heart rate and blood pressure (191) and considering sICAM-1's potential role as a biomarker for cardiovascular risk (192) these findings underscore the importance for further investigation into the cardiovascular implications of ADHD medication. Prior studies, including a Swedish register cohort study (26) have indicated elevated risk of pathological hypertension associated

with ADHD medication, underlining the need for a deeper investigation into potential mitigative strategies. We did find a suggestive synbiotic-specific reduction of sICAM-1 levels in children with psychostimulant medication. The possible use of Synbiotic2000 as an adjuvant treatment to ameliorate inflammatory processes and potentially reduce cardiovascular risk provides for a significant clinical interest. Notably, sICAM-1 has previously been associated with obesity (193) and depression (133), but we did not adjust for BMI in the child analysis, hence we cannot rule out that higher sICAM-1 levels in the child cohort were associated with higher BMI or depressed mood. However, we could exclude such confounding in the adult cohort. The co-occurance between ADHD, psychiatric disorders, GI symptoms (68-70) and uppregulated sICAM-1 suggests an interrelation (193). Further research into the complex interplay between SCFAs, immune markers, and ADHD pathology is crucial to unravel these mechanisms and their clinical implications.

Emotion dysregulation is a common feature in ADHD (4), further there is a high co-occurance between ASD and ADHD (194). In study I there was a significant treatment effect in emotional regulation and ASD symptoms in the ADHD cohort with higher proinflammatory levels at baseline. Emotion regulation difficulties are known to cause additional suffering in individuals with ADHD and neither ASD symptoms nor emotional regulatory difficulties are improved by standard stimulat treatment (191, 195) which in addition can have sideeffects (191). The Synbiotic2000 treatment was well tolerated and has potential effects, possibly through the gut-brain axis, on common co-occurring symptoms in ADHD, which poses a clinical interest.

In study III and IV we provide findings that support the notion that prenatal exposure to maternal ADs can predispose offspring to a range of mental health disorders, generally the risks were small and predominantly relevant at a public health level. A few AD exposures were associated with higher risks, still with modest effect sizes, which might propose that offspring to these certain maternal ADs might benefit from psychiatric monitoring to enable early interventions. Some studies suggest that probiotic interventions pre and/or post birth might reduce risk of NDDs in offspring. The evidence remains inconclusive, underscoring the need for further research (130, 138). Females with ADs tend to give birth to fewer children, which to some extent is explained by concerns about offspring health outcomes (196), further underlining the clinical relevance and need for accurate risk estimates, and when relevant, possible monotoring or preventive strategies.

5.4 Strenghts of the studies

In study I and II we integrate clinical assessments with biological markers, adopting a comprehensive approach that provides valuable and novel information. This methodology enabled us to investigate treatment effects in different stratas. An RCT design reduces bias, enhance reliabity of the results by randomly assigning participants to either the intervention or control group, thereby improving our ability to infer causality. One major strength in our RCT is the sample size, more than doubled the size compared to other studies, further the duration of the intervention is among the longest for probiotics, and the treatment being multiple bacterial strains including fibers is rarely used (140) and, to the best of our knowledge, it is still the first report of a comprehensive immune activity marker profiling in child and adult ADHD. Another strength is that the SCFA levels are measured in plasma, and lastly, we performed correlation analysis between immune system proteins and SCFAs. In study III and IV we used an epidemological design investigating prenatal exposure to maternal ADs. A strength of this approach lies in the use of large-scale national birth registry data, with over 1 million births, which offers robust and generalizable findings. Further the substantial poupulation characteristics, ranging from maternal psychotropics to offspring birthweight, enabled us to make thorough adjustments minimizing the risk of confunding. There is however, always the possiblity of unknown confounders that introduce biases or affect the accuracy of the findings. To address this, we performed several sensitivity analyses, e.g. perinatal risk factors, to support the validity of our findings. The diagnoses in our registry data have been validated, which increases the accuracy and reliability of our results and minimizes the risk of misclassification. Nevertheless, the immune system's role on offspring during pregnancy is intricate and not fully elucidated, especially regarding how specific ADs might influence fetal brain development. Identifying maternal conditions that increase the risk of psychiatric and NDDs in offspring could pave the way for targeted interventions and monitoring strategies, potentially diminishing these risks. Taken together, the findings reported in this thesis underscore the necessity for further research into the gut-brain axis and the prenatal immune environment's role in the etiology and manifestation of mental disorders. The limitations of this study are discussed in the respective sections above.

6 Conclusions

This thesis explores effects of (i) a synbiotic treatment in a ADHD cohort and (ii) prenatal exposure to maternal ADs on NDDs and psychiatric disorders. In study I we show treatment specific effects on (i) ASD and (ii) Emotion dysregulation symptoms in children and adults, respectively, in the ADHD cohort with higer levels of sVCAM-1 at baseline. In study II we show immune activity and SCFA plasma profiles in ADHD and a negative correlation between certain inflammatory markers and SCFAs, and a tendency of treatment specific effects on child propionic acid and certain proinflammatory markers, and treatment specific effects on IL-12/IL-23p40 in children with ADHD on stimulant medication. Using an epidemiological approach we identified increased risks for disorders such as sleep, eating, and incontinence disorders in offspring exposed to maternal CD (study III). And further we show risk estimates for a comprehensive range of maternal AD-offspring mental disorder associations, enabeling effect size comparisons (study IV). In study III and IV we show increased HR for several NDDs and psychiatric outcomes that is not fully explaind by adverse birth outcomes. In conclusion, this thesis underscores the complex interplay between immune function and mental health, spanning from prenatal development to childhood. However, futher reserch is needed to verify these results, preferably with a longitudinal approach to address causality.

7 Future perspectives

Inflammatory markers and synbiotic effects in ADHD

We demonstrate elevated concentrations of inflammatory markers, specifically sVCAM-1 and sICAM-1, in adults diagnosed with ADHD. This may represent an early indicator of a developing cardiovascular disorder (CVD), which has been reported to be overrepresented in ADHD population (197). Notably, these levels were even more elevated in children undergoing stimulant medication compared to their untreated counterparts. Further, we report that the cohort of children with higher baseline sVCAM-1 levels improved in ASD symptoms by oral synbiotic2000 treatment, and adults in emotion regulation. In addition, Synbiotic2000 significantly reduced IL-12/IL-23p40 in the children, and suggestively reduced sICAM-1 and sVCAM-1 as well as a few other markers relevant for vascular inflammation or angiogenesis. This may indicate that Synbiotic2000 is acting through an antiinflammatory pathway, particularly benefiting those with a higher inflammatory state at baseline, thereby potentially relevant for protecting against CVD development. However, there was not a clear overlap between markers elevated at baseline and those found to be changed by the synbiotic. Also, the effect variation between patient groups may be because of limited group sample sizes. It is unknown if there is a causal relationship between stimulant treatment and vascular inflammation. However, stimulant medication is known to often cause increased blood pressure (191), and is proposed to be associated with pathologic hypertension associated with the duration of treatment (26). To clarify these relationships, further longitudinal studies are necessary.

Current and future research directions on vascular effects of psychostimulants

Our reserch group has initiated a new longitudinal clinical observational study aiming to investigate the causative effect of psychostimulant medication in ADHD on the vascular wall. Treatment-naïve children with ADHD are invited to participate and blood samples are collected at several timepoints. As a second step in this longitudinal study, we will introduce an open-label Synbiotic treatment and monitor potential effects in plasma and the fecal microbiome. In parallell, we are investigating psychostimulant effects, as well as the putative SCFA-vascular inflammation interaction *in vitro,* in two human endothelial cell models.

Epidemiological insights into effects of maternal autoimmunity and offspring mental health risks

Further significant risks for offspring mental disorder, predominantly NDDs, were observed following exposure to various maternal ADs. These risks were not fully explained by perinatal risk factors. Notably, specific offspring disorders exhibited more than a two-fold increase in risk associated with certain ADs, such as sleep disorders in mothers with SIC, and autism in mothers with autoimmune thyroiditis. Previous findings of association between parental ADs and offspring mental disorders have suggested a stronger maternal contribution, implicating that *in utero* environmental factors might contribute to mental disorder, in addition to genetic factors (142, 143, 146, 151, 153).

Suggestions for future research

In future epidemiological research assessing the risk to offspring exposed to various maternal ADs, it would be valuable to explore maternal medication during pregnancy, potentially categorizing exposures into traditional AD pharmaceuticals and monoclonal antibodies and assess effects on offspring mental disorder. In addition, it would be interesting to further investigate if a gut-brain targeted treatment during pregnancy is beneficial for offspring health. Finally, while maternal psychostimulant medication during pregnancy does not seem to increase the risk for NDDs in the offspring (198), it would also be interesting to investigate the effect on cardiometabolic risk in the offspring.

Key findings and their impact

This thesis combines clinical and epidemiological findings, which, taken together proposes a gut-brain axis and immune activity involvement in prenatal neurodevelopment and NDDs and a potential novel adjuvant therapeutic in ADHD. While the implications of these findings are possibly significant for both clinical practice and public health policy, replication of results or support through mechanistic research is essential. From a clinical perspective, the use of synbiotic treatment could hold potential benefits for patients with ADHD, in managing symptoms such as emotional dysregulation and mitigating any, possibly medication associated, increases of inflammation in blood vessels. Understanding the impact of maternal health and implication on prenatal neurodevelopment could guide preventive strategies and interventions aimed at minimizing prenatal risks.

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9 References

1. Barkley RA, Dupaul GJ, McMurray MB. COMPREHENSIVE EVALUATION OF ATTENTION-DEFICIT DISORDER WITH AND WITHOUT HYPERACTIVITY AS DEFINED BY RESEARCH CRITERIA. Journal of Consulting and Clinical Psychology. 1990;58(6):775-89.
2. Conrad P, Potter D. From hyperactive children to ADHD adults: Observations on the expansion of medical categories. Soc Probl. 2000;47(4):559-82.
3. Faraone SV, Biederman J, Mick E. The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychological medicine. 2006;36(2):159-65.
4. Soler-Gutiérrez AM, Pérez-González JC, Mayas J. Evidence of emotion dysregulation as a core symptom of adult ADHD: A systematic review. PloS one. 2023;18(1).
5. Elia J, Capasso M, Zaheer Z, Lantieri F, Ambrosini P, Berrettini W, et al. Candidate gene analysis in an on-going genome-wide association study of attention-deficit hyperactivity disorder: suggestive association signals in ADRA1A. Psychiatric Genetics. 2009;19(3):134-41.
6. Neale BM, Medland S, Ripke S, Anney RJL, Asherson P, Buitelaar J, et al. Case-Control Genome-Wide Association Study of Attention-Deficit/Hyperactivity Disorder. J Am Acad Child Adolesc Psychiatr. 2010;49(9):906-20.
7. Stergiakouli E, Thapar A. Fitting the pieces together: current research on the genetic basis of attention-deficit/hyperactivity disorder (ADHD). Neuropsychiatric Disease and Treatment. 2010;6:551-60.
8. Brikell I, Burton C, Mota NR, Martin J. Insights into attention-deficit/hyperactivity disorder from recent genetic studies. Psychological medicine. 2021;51(13):2274-86.
9. Biederman J, Faraone SV. Attention-deficit hyperactivity disorder. Lancet (London, England). 2005;366(9481):237-48.
10. Luo YY, Weibman D, Halperin JM, Li XB. A Review of Heterogeneity in Attention Deficit/Hyperactivity Disorder (ADHD). Front Hum Neurosci. 2019;13:12.
11. Froehlich TE, Anixt JS, Loe IM, Chirdkiatgumchai V, Kuan L, Gilman RC. Update on Environmental Risk Factors for Attention-Deficit/Hyperactivity Disorder. Curr Psychiatry Rep. 2011;13(5):333-44.
12. Drechsler R, Brem S, Brandeis D, Gruenblatt E, Berger G, Walitza S. ADHD: Current Concepts and Treatments in Children and Adolescents. Neuropediatrics. 2020;51(05):315-35.
13. Yu M, Gao X, Niu X, Zhang M, Yang Z, Han S, et al. Meta-analysis of structural and functional alterations of brain in patients with attention-deficit/hyperactivity disorder. Front Psychiatry. 2022;13:1070142.
14. Posner J, Park C, Wang ZS. Connecting the Dots: A Review of Resting Connectivity MRI Studies in Attention-Deficit/Hyperactivity Disorder. Neuropsychology Review. 2014;24(1):3-15.

15. Saad JF, Griffiths KR, Korgaonkar MS. A Systematic Review of Imaging Studies in the Combined and Inattentive Subtypes of Attention Deficit Hyperactivity Disorder. Frontiers in Integrative Neuroscience. 2020;14.
16. Singh I. SCIENCE AND SOCIETY Beyond polemics: science and ethics of ADHD. Nat Rev Neurosci. 2008;9(12):957-64.
17. Kazda L, Bell K, Thomas R, McGeechan K, Sims R, Barratt A. Overdiagnosis of Attention-Deficit/Hyperactivity Disorder in Children and Adolescents A Systematic Scoping Review. JAMA Netw Open. 2021;4(4):29.
18. Willcutt E. The Prevalence of DSM-IV Attention-Deficit/Hyperactivity Disorder: A Meta-Analytic Review. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2012;9(3):490-9.
19. Polanczyk GV, Gwillcutt E, Salum GA, Kieling C, Rohde LA. ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. Int J Epidemiol. 2014;43(2):434-42.
20. Salari N, Ghasemi H, Abdoli N, Rahmani A, Shiri MH, Hashemian AH, et al. The global prevalence of ADHD in children and adolescents: a systematic review and meta-analysis. Italian Journal of Pediatrics. 2023;49(1).
21. Ayano G, Demelash S, Gizachew Y, Tsegay L, Alati R. The global prevalence of attention deficit hyperactivity disorder in children and adolescents: An umbrella review of meta-analyses. J Affect Disord. 2023;339:860-6.
22. London AS, Landes SD. Cohort Change in the Prevalence of ADHD Among US Adults: Evidence of a Gender-Specific Historical Period Effect. Journal of attention disorders. 2021;25(6):771-82.
23. Conrad P, Bergey MR. The impending globalization of ADHD: Notes on the expansion and growth of a medicalized disorder. Soc Sci Med. 2014;122:31-43.
24. Brikell I, Yao H, Li L, Astrup A, Gao L, Gillies MB, et al. ADHD medication discontinuation and persistence across the lifespan: a retrospective observational study using population-based databases. Lancet Psychiatry. 2024;11(1):16-26.
25. Storebo OJ, Pedersen N, Ramstad E, Kielsholm ML, Nielsen SS, Krogh HB, et al. Methylphenidate for attention deficit hyperactivity disorder (ADHD) in children and adolescents - assessment of adverse events in non-randomised studies. Cochrane Database Syst Rev. 2018(5):475.
26. Zhang L, Li L, Andell P, Garcia-Argibay M, Quinn PD, D'Onofrio BM, et al. Attention-Deficit/Hyperactivity Disorder Medications and Long-Term Risk of Cardiovascular Diseases. JAMA Psychiatry. 2024;81(2):178-87.
27. Zhang L, Yao H, Li L, Du Rietz E, Andell P, Garcia-Argibay M, et al. Risk of Cardiovascular Diseases Associated With Medications Used in Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-analysis. JAMA Netw Open. 2022;5(11):e2243597.
28. Boland H, DiSalvo M, Fried R, Woodworth KY, Wilens T, Faraone SV, et al. A literature review and meta-analysis on the effects of ADHD medications on functional outcomes. Journal of psychiatric research. 2020;123:21-30.

29. Chang Z, Ghirardi L, Quinn PD, Asherson P, D'Onofrio BM, Larsson H. Risks and Benefits of Attention-Deficit/Hyperactivity Disorder Medication on Behavioral and Neuropsychiatric Outcomes: A Qualitative Review of Pharmacoepidemiology Studies Using Linked Prescription Databases. Biological Psychiatry. 2019;86(5):335-43.
30. Riera M, Castells X, Tobias A, Cunill R, Blanco L, Capella D. Discontinuation of pharmacological treatment of children and adolescents with attention deficit hyperactivity disorder: meta-analysis of 63 studies enrolling 11,788 patients. Psychopharmacology. 2017;234(17):2657-71.
31. Morris-Rosendahl DJ, Crocq MA. Neurodevelopmental disorders-the history and future of a diagnostic concept. Dialogues in Clinical Neuroscience. 2020;22(1):65-72.
32. Mattheisen M, Grove J, Als TD, Martin J, Voloudakis G, Meier S, et al. Identification of shared and differentiating genetic architecture for autism spectrum disorder, attention-deficit hyperactivity disorder and case subgroups. Nature Genetics. 2022;54(10):1470-+.
33. Zeidan J, Fombonne E, Scorah J, Ibrahim A, Durkin MS, Saxena S, et al. Global prevalence of autism: A systematic review update. Autism Research. 2022;15(5):778-90.
34. Rong Y, Yang CJ, Jin Y, Wang Y. Prevalence of attention-deficit/hyperactivity disorder in individuals with autism spectrum disorder: A meta-analysis. Res Autism Spectr Disord. 2021;83:13.
35. Hollingdale J, Woodhouse E, Young S, Fridman A, Mandy W. Autistic spectrum disorder symptoms in children and adolescents with attention-deficit/hyperactivity disorder: a meta-analytical review. Psychological medicine. 2020;50(13):2240-53.
36. Bölte S, Girdler S, Marschik PB. The contribution of environmental exposure to the etiology of autism spectrum disorder. Cellular and Molecular Life Sciences. 2019;76(7):1275-97.
37. Sauer AK, Stanton JE, Hans S, Grabrucker AM. Autism Spectrum Disorders: Etiology and Pathology. In: Grabrucker AM, editor. Autism Spectrum Disorders. Brisbane (AU): Exon Publications
Copyright: The Authors.; 2021.
38. Gidziela A, Ahmadzadeh YI, Michelini G, Allegrini AG, Agnew-Blais J, Lau LY, et al. A meta-analysis of genetic effects associated with neurodevelopmental disorders and co-occurring conditions. Nature Human Behaviour. 2023;7(4):642-56.
39. Leffa DT, Torres ILS, Rohde LA. A Review on the Role of Inflammation in Attention-Deficit/Hyperactivity Disorder. Neuroimmunomodulation. 2018;25(5-6):328-33.
40. Sanlier N, Kocabas S. The effect of probiotic, prebiotic and gut microbiota on ASD: A review and future perspectives. Critical Reviews in Food Science and Nutrition. 2023;63(15):2319-30.
41. Feng PY, Zhao S, Zhang YY, Li EY. A review of probiotics in the treatment of autism spectrum disorders: Perspectives from the gut-brain axis. Frontiers in Microbiology. 2023;14.

42. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nature Medicine. 2019;25(12):1822-32.
43. Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes. 2012;3(1):4-14.
44. Kelly JR, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbiota axis: challenges for translation in psychiatry. Annals of Epidemiology. 2016;26(5):366-72.
45. Fung TC, Olson CA, Hsiao EY. Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci. 2017;20(2):145-55.
46. Dubois T, Reynaert C, Jacques D, Lepiece B, Patigny P, Zdanowicz N. IMMUNITY AND PSYCHIATRIC DISORDERS: VARIABILITIES OF IMMUNITY BIOMARKERS ARE THEY SPECIFIC? Psychiatria Danubina. 2018;30:447-51.
47. Cai HQ, Catts VS, Webster MJ, Galletly C, Liu D, O'Donnell M, et al. Increased macrophages and changed brain endothelial cell gene expression in the frontal cortex of people with schizophrenia displaying inflammation. Molecular psychiatry. 2020;25(4):761-75.
48. Feigenson KA, Kusnecov AW, Silverstein SM. Inflammation and the two-hit hypothesis of schizophrenia. Neuroscience and Biobehavioral Reviews. 2014;38:72-93.
49. Stertz L, Magalhaes PVS, Kapczinski F. Is bipolar disorder an inflammatory condition? The relevance of microglial activation. Current Opinion in Psychiatry. 2013;26(1):19-26.
50. Derry HM, Padin AC, Kuo JL, Hughes S, Kiecolt-Glaser JK. Sex Differences in Depression: Does Inflammation Play a Role? Curr Psychiatry Rep. 2015;17(10).
51. Han QQ, Yu J. Inflammation: a mechanism of depression? Neuroscience Bulletin. 2014;30(3):515-23.
52. Niles AN, Smirnova M, Lin J, O'Donovan A. Gender differences in longitudinal relationships between depression and anxiety symptoms and inflammation in the health and retirement study. Psychoneuroendocrinology. 2018;95:149-57.
53. Doria A, Zen M, Bettio S, Gatto M, Bassi N, Nalotto L, et al. Autoinflammation and autoimmunity: Bridging the divide. Autoimmunity Reviews. 2012;12(1):22-30.
54. Pisetsky DS. Pathogenesis of autoimmune disease. Nat Rev Nephrol. 2023;19(8):509-24.
55. Chang JT. Pathophysiology of Inflammatory Bowel Diseases. New England Journal of Medicine. 2020;383(27):2652-64.
56. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018;11(1):1-10.
57. Conrad N, Misra S, Verbakel JY, Verbeke G, Molenberghs G, Taylor PN, et al. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort

study of 22 million individuals in the UK. Lancet (London, England). 2023;401(10391):1878-90.
58. Gillberg C, Gillberg IC, Rasmussen P, Kadesjo B, Soderstrom H, Rastam M, et al. Co-existing disorders in ADHD - implications for diagnosis and intervention. Eur Child Adolesc Psych. 2004;13:80-92.
59. Gnanavel S, Sharma P, Kaushal P, Hussain S. Attention deficit hyperactivity disorder and comorbidity: A review of literature. World J Clin Cases. 2019;7(17):2420-6.
60. Brevik EJ, Lundervold AJ, Halmoy A, Posserud MB, Instanes JT, Bjorvatn B, et al. Prevalence and clinical correlates of insomnia in adults with attention-deficit hyperactivity disorder. Acta Psychiatrica Scandinavica. 2017;136(2):220-7.
61. Barberio B, Zamani M, Black CJ, Savarino EV, Ford AC. Prevalence of symptoms of anxiety and depression in patients with inflammatory bowel disease: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2021;6(5):359-70.
62. Bisgaard TH, Allin KH, Keefer L, Ananthakrishnan AN, Jess T. Depression and anxiety in inflammatory bowel disease: epidemiology, mechanisms and treatment. Nature Reviews Gastroenterology & Hepatology. 2022;19(11):717-26.
63. Bernstein CN, Hitchon CA, Walld R, Bolton JM, Sareen J, Walker JR, et al. Increased Burden of Psychiatric Disorders in Inflammatory Bowel Disease. Inflammatory Bowel Diseases. 2019;25(2):360-8.
64. Arp L, Jansson S, Wewer V, Burisch J. Psychiatric Disorders in Adult and Paediatric Patients With Inflammatory Bowel Diseases - A Systematic Review and Meta-Analysis. J Crohns Colitis. 2022;16(12):1933-45.
65. Yanartas O, Kani HT, Bicakci E, Kilic I, Banzragch M, Acikel C, et al. The effects of psychiatric treatment on depression, anxiety, quality of life, and sexual dysfunction in patients with inflammatory bowel disease. Neuropsychiatr Dis Treat. 2016;12:673-83.
66. Barberio B, Zamani M, Black CJ, Savarino EV, Ford AC. Prevalence of symptoms of anxiety and depression in patients with inflammatory bowel disease: a systematic review and meta-analysis. Lancet Gastroenterology & Hepatology. 2021;6(5):359-70.
67. Wang JY, Ma B, Wang JJ, Zhang ZY, Chen O. Global prevalence of autism spectrum disorder and its gastrointestinal symptoms: A systematic review and meta-analysis. Front Psychiatry. 2022;13.
68. Ming X, Chen N, Ray C, Brewer G, Kornitzer J, Steer RA. A Gut Feeling: A Hypothesis of the Role of the Microbiome in Attention-Deficit/Hyperactivity Disorders. Child Neurol Open. 2018;5:2329048x18786799.
69. Kedem S, Yust-Katz S, Carter D, Levi Z, Kedem R, Dickstein A, et al. Attention deficit hyperactivity disorder and gastrointestinal morbidity in a large cohort of young adults. World journal of gastroenterology. 2020;26(42):13.
70. McKeown C, Hisle-Gorman E, Eide M, Gorman GH, Nylund CM. Association of Constipation and Fecal Incontinence With Attention-Deficit/Hyperactivity Disorder. Pediatrics. 2013;132(5):E1210-E5.

71. Shah E, Rezaie A, Riddle M, Pimentel M. Psychological disorders in gastrointestinal disease: epiphenomenon, cause or consequence? Ann Gastroenterol. 2014;27(3):224-30.
72. Pan PY, Bolte S. The association between ADHD and physical health: a co-twin control study. Sci Rep. 2020;10(1):13.
73. Miller I. The gut-brain axis: historical reflections. Microbial ecology in health and disease. 2018;29(1):1542921.
74. Track NS. The gastrointestinal endocrine system. Canadian Medical Association journal. 1980;122(3):287-92.
75. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol. 2015;28(2):203-9.
76. Brussow H. Problems with the concept of gut microbiota dysbiosis. Microbial Biotechnology. 2020;13(2):423-34.
77. Arneth BM. Gut-brain axis biochemical signalling from the gastrointestinal tract to the central nervous system: gut dysbiosis and altered brain function. Postgrad Med J. 2018;94(1114):446-52.
78. Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Front Endocrinol. 2020;11:14.
79. Tosoni G, Conti M, Heijtz RD. Bacterial peptidoglycans as novel signaling molecules from microbiota to brain. Current Opinion in Pharmacology. 2019;48:107-13.
80. Swann JR, Heijtz RD, Mayneris-Perxachs J, Arora A, Isaksson J, Bölte S, et al. Characterizing the metabolomic signature of attention-deficit hyperactivity disorder in twins. Neuropharmacology. 2023;234.
81. Swann JR, Spitzer SO, Diaz Heijtz R. Developmental Signatures of Microbiota-Derived Metabolites in the Mouse Brain. Metabolites. 2020;10(5).
82. Heijtza RD, Wang SG, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A. 2011;108(7):3047-52.
83. Eltokhi A, Janmaat IE, Genedi M, Haarman BCM, Sommer IEC. Dysregulation of synaptic pruning as a possible link between intestinal microbiota dysbiosis and neuropsychiatric disorders. J Neurosci Res. 2020;98(7):1335-69.
84. Erny D, de Angelis ALH, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18(7):965-+.
85. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. World journal of gastroenterology. 2015;21(29):8787-803.
86. Ikeda-Ohtsubo W, Brugman S, Warden CH, Rebel JMJ, Folkerts G, Pieterse CMJ. How Can We Define "Optimal Microbiota?": A Comparative Review of Structure and Functions of Microbiota of Animals, Fish, and Plants in Agriculture. Frontiers in Nutrition. 2018;5.
87. Ferrer M, Mendez-Garcia C, Rojo D, Barbas C, Moya A. Antibiotic use and microbiome function. Biochemical Pharmacology. 2017;134:114-26.

88. Lurie I, Yang YX, Haynes K, Mamtani R, Boursi B. Antibiotic Exposure and the Risk for Depression, Anxiety, or Psychosis: A Nested Case-Control Study. J Clin Psychiatry. 2015;76(11):1522-+.
89. Mathee K, Cickovski T, Deoraj A, Stollstorff M, Narasimhan G. The gut microbiome and neuropsychiatric disorders: implications for attention deficit hyperactivity disorder (ADHD). J Med Microbiol. 2020;69(1):14-24.
90. Hamad AF, Alessi-Severini S, Mahmud SM, Brownell M, Kuo IF. Prenatal antibiotics exposure and the risk of autism spectrum disorders: A population-based cohort study. PloS one. 2019;14(8):13.
91. Gibson GR, Roberfroid MB. DIETARY MODULATION OF THE HUMAN COLONIC MICROBIOTA - INTRODUCING THE CONCEPT OF PREBIOTICS. Journal of Nutrition. 1995;125(6):1401-12.
92. Markowiak P, Slizewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients. 2017;9(9).
93. Springer TA. ADHESION RECEPTORS OF THE IMMUNE-SYSTEM. Nature. 1990;346(6283):425-34.
94. Yuan N, Chen Y, Xia Y, Dai JC, Liu CY. Inflammation-related biomarkers in major psychiatric disorders: a cross-disorder assessment of reproducibility and specificity in 43 meta-analyses. Transl Psychiatr. 2019;9.
95. Müller N. The Role of Intercellular Adhesion Molecule-1 in the Pathogenesis of Psychiatric Disorders. Frontiers in Pharmacology. 2019;10.
96. Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S, et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nature Communications. 2015;6.
97. Zhang D, Jian YP, Zhang YN, Li Y, Gu LT, Sun HH, et al. Short-chain fatty acids in diseases. Cell Commun Signal. 2023;21(1):212.
98. Luczynski P, McVey Neufeld KA, Oriach CS, Clarke G, Dinan TG, Cryan JF. Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. Int J Neuropsychopharmacol. 2016;19(8).
99. Desbonnet L, Clarke G, Traplin A, O'Sullivan O, Crispie F, Moloney RD, et al. Gut microbiota depletion from early adolescence in mice: Implications for brain and behaviour. Brain, behavior, and immunity. 2015;48:165-73.
100. Cryan JF, Dinan TG. More than a gut feeling: the microbiota regulates neurodevelopment and behavior. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2015;40(1):241-2.
101. Morel C, Sanchez IM, Cherifi Y, Chartrel N, Heijtz RD. Perturbation of maternal gut microbiota in mice during a critical perinatal window influences early neurobehavioral outcomes in offspring. Neuropharmacology. 2023;229.
102. Kelly JR, Borre Y, Brien CO, Patterson E, El Aidy S, Deane J, et al. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. Journal of psychiatric research. 2016;82:109-18.

103. Zheng P, Zeng BH, Liu ML, Chen JJ, Pan JX, Han Y, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. Science Advances. 2019;5(2).
104. Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell. 2019;177(6):1600-+.
105. Tengeler AC, Dam SA, Wiesmann M, Naaijen J, van Bodegom M, Belzer C, et al. Gut microbiota from persons with attention-deficit/hyperactivity disorder affects the brain in mice. Microbiome. 2020;8(1):44.
106. Quagliato LA, de Matos U, Nardi AE. Maternal immune activation generates anxiety in offspring: A translational meta-analysis. Transl Psychiatry. 2021;11(1):245.
107. Tremblay A, Lingrand L, Maillard M, Feuz B, Tompkins TA. The effects of psychobiotics on the microbiota-gut-brain axis in early-life stress and neuropsychiatric disorders. Prog Neuro-Psychopharmacol Biol Psychiatry. 2021;105:10.
108. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimaki M. Cumulative meta-analysis of interleukins 6 and 1 beta, tumour necrosis factor alpha and C-reactive protein in patients with major depressive disorder. Brain Behavior and Immunity. 2015;49:206-15.
109. Krysta K, Krzystanek M, Bratek A, Krupka-Matuszczyk I. Sleep and inflammatory markers in different psychiatric disorders. J Neural Transm. 2017;124:S179-S86.
110. Han VX, Patel S, Jones HF, Nielsen TC, Mohammad SS, Hofer MJ, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. Transl Psychiatr. 2021;11(1).
111. Steel DM, Whitehead AS. THE MAJOR ACUTE-PHASE REACTANTS - C-REACTIVE PROTEIN, SERUM AMYLOID-P COMPONENT AND SERUM AMYLOID-A PROTEIN. Immunology Today. 1994;15(2):81-8.
112. Andreo-Martinez P, Rubio-Aparicio M, Sanchez-Meca J, Veas A, Martinez-Gonzalez AE. A Meta-analysis of Gut Microbiota in Children with Autism. Journal of autism and developmental disorders. 2021:14.
113. Stiernborg M, Debelius JW, Yang LL, Skott E, Millischer V, Giacobini M, et al. Bacterial gut microbiome differences in adults with ADHD and in children with ADHD on psychostimulant medication. Brain Behavior and Immunity. 2023;110:310-21.
114. Hiergeist A, Gessner J, Gessner A. Current Limitations for the Assessment of the Role of the Gut Microbiome for Attention Deficit Hyperactivity Disorder (ADHD). Front Psychiatry. 2020;11.
115. Sukmajaya AC, Lusida MI, Soetjipto, Setiawati Y. Systematic review of gut microbiota and attention-deficit hyperactivity disorder (ADHD). Ann Gen Psychiatry. 2021;20(1):12.

116. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nature Reviews Gastroenterology & Hepatology. 2019;16(8):461-78.
117. Boonchooduang N, Louthrenoo O, Likhitweerawong N, Thonusin C, Chattipakorn N, Chattipakorn SC. Fecal Short-Chain Fatty Acids as Potential Biomarkers for Attention-Deficit/Hyperactivity Disorder. Eur Psychiat. 2023;66:S393-S4.
118. Tostes M, Teixeira HC, Gattaz WF, Brandao MAF, Raposo NRB. Altered Neurotrophin, Neuropeptide, Cytokines and Nitric Oxide Levels in Autism. Pharmacopsychiatry. 2012;45(6):241-3.
119. Zerbo O, Yoshida C, Grether JK, Van de Water J, Ashwood P, Delorenze GN, et al. Neonatal cytokines and chemokines and risk of Autism Spectrum Disorder: the Early Markers for Autism (EMA) study: a case-control study. J Neuroinflamm. 2014;11.
120. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, de Water JV. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behavior and Immunity. 2011;25(1):40-5.
121. Yamauchi T, Makinodan M, Toritsuka M, Okumura K, Kayashima Y, Ishida R, et al. Tumor necrosis factor-alpha expression aberration of M1/M2 macrophages in adult high-functioning autism spectrum disorder. Autism Research. 2021.
122. Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM, Buie TM, et al. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. Molecular Autism. 2016;7.
123. Mitchell RHB, Goldstein BI. Inflammation in Children and Adolescents With Neuropsychiatric Disorders: A Systematic Review. J Am Acad Child Adolesc Psychiatr. 2014;53(3):274-96.
124. Galvez-Contreras AY, Campos-Ordonez T, Gonzalez-Castaneda RE, Gonzalez-Perez O. Alterations of Growth Factors in Autism and Attention-Deficit/Hyperactivity Disorder. Front Psychiatry. 2017;8.
125. Ferencova N, Visnovcova Z, Ondrejka I, Hrtanek I, Bujnakova I, Kovacova V, et al. Peripheral Inflammatory Markers in Autism Spectrum Disorder and Attention Deficit/Hyperactivity Disorder at Adolescent Age. International journal of molecular sciences. 2023;24(14).
126. Misiak B, Wójta-Kempa M, Samochowiec J, Schiweck C, Aichholzer M, Reif A, et al. Peripheral blood inflammatory markers in patients with attention deficit/hyperactivity disorder (ADHD): A systematic review and meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry. 2022;118:110581.
127. Sullivan EL, Riper KM, Lockard R, Valleau JC. Maternal high-fat diet programming of the neuroendocrine system and behavior. Hormones and Behavior. 2015;76:153-61.
128. Kong LH, Norstedt G, Schalling M, Gissler M, Lavebratt C. The Risk of Offspring Psychiatric Disorders in the Setting of Maternal Obesity and Diabetes. Pediatrics. 2018;142(3).

129. Nielsen TC, Nassar N, Shand AW, Jones HF, Han VX, Patel S, et al. Association between cumulative maternal exposures related to inflammation and child attention-deficit/hyperactivity disorder: A cohort study. Paediatr Perinat Epidemiol. 2024;38(3):241-50.
130. Pärtty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatr Res. 2015;77(6):823-8.
131. Lavebratt C, Yang LL, Giacobini M, Forsell Y, Schalling M, Partonen T, et al. Early exposure to antibiotic drugs and risk for psychiatric disorders: a population-based study. Transl Psychiatr. 2019;9:12.
132. Anand D, Colpo GD, Zeni G, Zeni CP, Teixeira AL. Attention-Deficit/Hyperactivity Disorder And Inflammation: What Does Current Knowledge Tell Us? A Systematic Review. Front Psychiatry. 2017;8.
133. Muller N. The Role of Intercellular Adhesion Molecule-1 in the Pathogenesis of Psychiatric Disorders. Frontiers in Pharmacology. 2019;10.
134. Alasehirli B, Oguz E, Gokcen C, Erbagci AB, Orkmez M, Demiryurek AT. Relationship between soluble intercellular adhesion molecules and attention-deficit/hyperactivity disorder. International Journal of Psychiatry in Medicine. 2015;50(2):238-47.
135. Asbjornsdottir B, Snorradottir H, Andresdottir E, Fasano A, Lauth B, Gudmundsson LS, et al. Zonulin-Dependent Intestinal Permeability in Children Diagnosed with Mental Disorders: A Systematic Review and Meta-Analysis. Nutrients. 2020;12(7):27.
136. Sandhu KV, Sherwin E, Schellekens H, Stanton C, Dinan TG, Cryan JF. Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. Transl Res. 2017;179:223-44.
137. He X, Liu WX, Tang FR, Chen X, Song GR. Effects of Probiotics on Autism Spectrum Disorder in Children: A Systematic Review and Meta-Analysis of Clinical Trials. Nutrients. 2023;15(6).
138. Slykerman RF, Kang J, Van Zyl N, Barthow C, Wickens K, Stanley T, et al. Effect of early probiotic supplementation on childhood cognition, behaviour and mood a randomised, placebo-controlled trial. Acta paediatrica (Oslo, Norway : 1992). 2018;107(12):2172-8.
139. Song WJ, Zhang M, Teng LL, Wang Y, Zhu LW. Prebiotics and probiotics for autism spectrum disorder: a systematic review and meta-analysis of controlled clinical trials. J Med Microbiol. 2022;71(4).
140. Liang SC, Sun CK, Chang CH, Cheng YS, Tzang RF, Chiu HJ, et al. Therapeutic efficacy of probiotics for symptoms of attention-deficit hyperactivity disorder in children and adolescents: meta-analysis. Bjpsych Open. 2024;10(1).
141. Mataix-Cols D, Frans E, Pérez-Vigil A, Kuja-Halkola R, Gromark C, Isomura K, et al. A total-population multigenerational family clustering study of autoimmune diseases in obsessive-compulsive disorder and Tourette's/chronic tic disorders. Molecular psychiatry. 2018;23(7):1652-8.

142. Nielsen PR, Benros ME, Dalsgaard S. Associations Between Autoimmune Diseases and Attention-Deficit/Hyperactivity Disorder: A Nationwide Study. J Am Acad Child Adolesc Psychiatry. 2017;56(3):234-40.e1.
143. Sun CK, Cheng YS, Chen IW, Chiu HJ, Chung W, Tzang RF, et al. Impact of parental rheumatoid arthritis on risk of autism spectrum disorders in offspring: A systematic review and meta-analysis. Front Med (Lausanne). 2022;9:1052806.
144. Yin W, Norrbäck M, Levine SZ, Rivera N, Buxbaum JD, Zhu H, et al. Maternal rheumatoid arthritis and risk of autism in the offspring. Psychological medicine. 2023:1-9.
145. Rom AL, Wu CS, Olsen J, Jawaheer D, Hetland ML, Mørch LS. Parental Rheumatoid Arthritis and Autism Spectrum Disorders in Offspring: A Danish Nationwide Cohort Study. J Am Acad Child Adolesc Psychiatry. 2018;57(1):28-32.e1.
146. Chen SY, Zhao SX, Dalman C, Karlsson H, Gardner R. Association of maternal diabetes with neurodevelopmental disorders: autism spectrum disorders, attention-deficit/hyperactivity disorder and intellectual disability. Int J Epidemiol. 2021;50(2):459-74.
147. Tsai PH, Yu KH, Chou IJ, Luo SF, Tseng WY, Huang LH, et al. Risk of autism spectrum disorder in children born to mothers with systemic lupus erythematosus and rheumatoid arthritis in Taiwan. Joint Bone Spine. 2018;85(5):599-603.
148. Vinet É, Pineau CA, Clarke AE, Scott S, Fombonne É, Joseph L, et al. Increased Risk of Autism Spectrum Disorders in Children Born to Women With Systemic Lupus Erythematosus: Results From a Large Population-Based Cohort. Arthritis Rheumatol. 2015;67(12):3201-8.
149. Shi LY, Lin LF. The trim-and-fill method for publication bias: practical guidelines and recommendations based on a large database of meta-analyses. Medicine. 2019;98(23).
150. Ellul P, Acquaviva E, Peyre H, Rosenzwajg M, Gressens P, Klatzmann D, et al. Parental autoimmune and autoinflammatory disorders as multiple risk factors for common neurodevelopmental disorders in offspring: a systematic review and meta-analysis. Transl Psychiatry. 2022;12(1):112.
151. He H, Yu YF, Liew ZY, Gissler M, Laszlo KD, Valdimarsdottir UA, et al. Association of Maternal Autoimmune Diseases With Risk of Mental Disorders in Offspring in Denmark. JAMA Netw Open. 2022;5(4).
152. Spann MN, Timonen-Soivio L, Suominen A, Cheslack-Postava K, McKeague IW, Sourander A, et al. Proband and Familial Autoimmune Diseases Are Associated With Proband Diagnosis of Autism Spectrum Disorders. J Am Acad Child Adolesc Psychiatry. 2019;58(5):496-505.
153. Hegvik TA, Chen Q, Kuja-Halkola R, Klungsøyr K, Butwicka A, Lichtenstein P, et al. Familial co-aggregation of attention-deficit/hyperactivity disorder and autoimmune diseases: a cohort study based on Swedish population-wide registers. Int J Epidemiol. 2022;51(3):898-909.
154. Rayes N, Seehofer D, Theruvath T, Mogl M, Langrehr JM, Nüssler NC, et al. Effect of enteral nutrition and synbiotics on bacterial infection rates after

pylorus-preserving pancreatoduodenectomy: a randomized, double-blind trial. Ann Surg. 2007;246(1):36-41.
155. Rayes N, Seehofer D, Hansen S, Boucsein K, Müller AR, Serke S, et al. Early enteral supply of lactobacillus and fiber versus selective bowel decontamination: a controlled trial in liver transplant recipients. Transplantation. 2002;74(1):123-7.
156. Plaudis H, Pupelis G, Zeiza K, Boka V. Early low volume oral synbiotic/prebiotic supplemented enteral stimulation of the gut in patients with severe acute pancreatitis: a prospective feasibility study. Acta Chir Belg. 2012;112(2):131-8.
157. Giamarellos-Bourboulis EJ, Bengmark S, Kanellakopoulou K, Kotzampassi K. Pro- and synbiotics to control inflammation and infection in patients with multiple injuries. J Trauma. 2009;67(4):815-21.
158. Vidot H, Cvejic E, Finegan LJ, Shores EA, Bowen DG, Strasser SI, et al. Supplementation with Synbiotics and/or Branched Chain Amino Acids in Hepatic Encephalopathy: A Pilot Randomised Placebo-Controlled Clinical Study. Nutrients. 2019;11(8).
159. Spindler-Vesel A, Bengmark S, Vovk I, Cerovic O, Kompan L. Synbiotics, prebiotics, glutamine, or peptide in early enteral nutrition: a randomized study in trauma patients. JPEN J Parenter Enteral Nutr. 2007;31(2):119-26.
160. Koutelidakis IM, Bezirtzoglou E, Giamarellos-Bourboulis EJ, Grosomanidis V, Kotzampassi K. Impact of synbiotics on the intestinal flora of critically ill patients with multiple injuries. Int J Antimicrob Agents. 2010;36(1):90-1.
161. Linetzky Waitzberg D, Alves Pereira CC, Logullo L, Manzoni Jacintho T, Almeida D, Teixeira da Silva ML, et al. Microbiota benefits after inulin and partially hydrolized guar gum supplementation: a randomized clinical trial in constipated women. Nutr Hosp. 2012;27(1):123-9.
162. Kolida S, Meyer D, Gibson GR. A double-blind placebo-controlled study to establish the bifidogenic dose of inulin in healthy humans. Eur J Clin Nutr. 2007;61(10):1189-95.
163. Kautto E, Ivarsson A, Norström F, Högberg L, Carlsson A, Hörnell A. Nutrient intake in adolescent girls and boys diagnosed with coeliac disease at an early age is mostly comparable to their non-coeliac contemporaries. J Hum Nutr Diet. 2014;27(1):41-53.
164. Han J, Lin K, Sequeira C, Borchers CH. An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography–tandem mass spectrometry. Anal Chim Acta. 2015;854:86-94.
165. Van Breukelen GJ. ANCOVA versus change from baseline: more power in randomized studies, more bias in nonrandomized studies [corrected]. J Clin Epidemiol. 2006;59(9):920-5.
166. Yang LL, Stiernborg M, Skott E, Söderström Å, Giacobini M, Lavebratt C. Proinflammatory mediators and their associations with medication and

comorbid traits in children and adults with ADHD. Eur Neuropsychopharmacol. 2020;41:118-31.
167. Skott E, Yang LL, Stiernborg M, Soderstrom A, Ruegg J, Schalling M, et al. Effects of a synbiotic on symptoms, and daily functioning in attention deficit hyperactivity disorder - A double-blind randomized controlled. Brain Behavior and Immunity. 2020;89:9-19.
168. Yang LL, Stiernborg M, Skott E, Xu JJ, Wu YJ, Landberg R, et al. Effects of a Synbiotic on Plasma Immune Activity Markers and Short-Chain Fatty Acids in Children and Adults with ADHD-A Randomized Controlled Trial. Nutrients. 2023;15(5).
169. <Pages_from_CY_BPGs_454-463.pdf>.
170. Cortese S. The Association between ADHD and Obesity: Intriguing, Progressively More Investigated, but Still Puzzling. Brain Sci. 2019;9(10).
171. Wu S, Ding Y, Wu F, Li R, Xie G, Hou J, et al. Family history of autoimmune diseases is associated with an increased risk of autism in children: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2015;55:322-32.
172. Inga Jácome MC, Morales Chacòn LM, Vera Cuesta H, Maragoto Rizo C, Whilby Santiesteban M, Ramos Hernandez L, et al. Peripheral Inflammatory Markers Contributing to Comorbidities in Autism. Behav Sci (Basel). 2016;6(4).
173. Bedrossian N, Haidar M, Fares J, Kobeissy FH, Fares Y. Inflammation and Elevation of Interleukin-12p40 in Patients with Schizophrenia. Frontiers in Molecular Neuroscience. 2016;9.
174. Moschen AR, Tilg H, Raine T. IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. Nature Reviews Gastroenterology & Hepatology. 2019;16(3):185-96.
175. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. Molecular psychiatry. 2016;21(12):1696-709.
176. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatrica Scandinavica. 2017;135(5):373-87.
177. Zhao HY, Zhang HQ, Liu SJ, Luo WL, Jiang YF, Gao JW. Association of Peripheral Blood Levels of Cytokines With Autism Spectrum Disorder: A Meta-Analysis. Front Psychiatry. 2021;12.
178. Yang LL, Stiernborg M, Skott E, Gillberg T, Landberg R, Giacobini M, et al. Lower plasma concentrations of short-chain fatty acids (SCFAs) in patients with ADHD. Journal of psychiatric research. 2022;156:36-43.
179. Plouet J, Bayard F. REGULATIONS OF VASCULATROPIN VASCULAR ENDOTHELIAL GROWTH-FACTOR BIOAVAILABILITY. Hormone Research. 1994;42(1-2):14-9.
180. Carvalho JF, Blank M, Shoenfeld Y. Vascular endothelial growth factor (VEGF) in autoimmune diseases. Journal of Clinical Immunology. 2007;27(3):246-56.
181. Rezazadeh L, Pourmoradian S, Tutunchi H, Farrin N, Radkhah N, Ostadrahimi A. The effects of probiotics on VCAM-1 and ICAM-1: A systematic

review and meta-analysis of randomized controlled trials. Clinical Nutrition Espen. 2023;54:60-7.
182. Ke S, Guimond AJ, Tworoger SS, Huang T, Chan AT, Liu YY, et al. Gut feelings: associations of emotions and emotion regulation with the gut microbiome in women. Psychological medicine. 2023;53(15):7151-60.
183. Toyoshima H, Yamamoto N, Komiya S. Letter to the editor: Gut feelings: associations of emotions and emotion regulation with the gut microbiome in women. Psychological medicine. 2024.
184. Ellul P, Acquaviva E, Peyre H, Rosenzwajg M, Gressens P, Klatzmann D, et al. Parental autoimmune and autoinflammatory disorders as multiple risk factors for common neurodevelopmental disorders in offspring: a systematic review and meta-analysis. Transl Psychiatr. 2022;12(1).
185. Jølving LR, Nielsen J, Beck-Nielsen SS, Nielsen RG, Friedman S, Kesmodel US, et al. The Association Between Maternal Chronic Inflammatory Bowel Disease and Long-term Health Outcomes in Children-A Nationwide Cohort Study. Inflamm Bowel Dis. 2017;23(8):1440-6.
186. Sadik A, Dardani C, Pagoni P, Havdahl A, Stergiakouli E, Khandaker GM, et al. Parental inflammatory bowel disease and autism in children. Nat Med. 2022;28(7):1406-11.
187. Adibi JJ, Zhao YQ, Koistinen H, Mitchell RT, Barrett ES, Miller R, et al. Molecular pathways in placental-fetal development and disruption. Molecular and Cellular Endocrinology. 2024;581.
188. Zuñiga LFF, Muñoz YS, Pustovrh MC. Thyroid hormones: Metabolism and transportation in the fetoplacental unit. Molecular Reproduction and Development. 2022;89(11):526-39.
189. Cruz-Rodríguez J, Díaz-López A, Canals-Sans J, Arija V. Maternal Vitamin B12 Status during Pregnancy and Early Infant Neurodevelopment: The ECLIPSES Study. Nutrients. 2023;15(6).
190. Nguyen V, Wuebbolt D, Pagnoux C, D'Souza R. Pregnancy outcomes in women with primary systemic vasculitis: a retrospective study. Journal of Maternal-Fetal & Neonatal Medicine. 2021;34(17):2771-7.
191. Nanda A, Janga LSN, Sambe HG, Yasir M, Man RK, Gogikar A, et al. Adverse Effects of Stimulant Interventions for Attention Deficit Hyperactivity Disorder (ADHD): A Comprehensive Systematic Review. Cureus Journal of Medical Science. 2023;15(9).
192. Singh V, Kaur R, Kumari P, Pasricha C, Singh R. ICAM-1 and VCAM-1: Gatekeepers in various inflammatory and cardiovascular disorders. Clinica Chimica Acta. 2023;548.
193. Mulhem A, Moulla Y, Klöting N, Ebert T, Tönjes A, Fasshauer M, et al. Circulating cell adhesion molecules in metabolically healthy obesity. International Journal of Obesity. 2021;45(2):331-6.
194. Francés L, Quintero J, Fernández A, Ruiz A, Caules J, Fillon G, et al. Current state of knowledge on the prevalence of neurodevelopmental disorders in childhood according to the DSM-5: a systematic review in accordance with the PRISMA criteria. Child and Adolescent Psychiatry and Mental Health. 2022;16(1).

195. Ventura P, de Giambattista C, Spagnoletta L, Trerotoli P, Cavone M, Di Gioia A, et al. Methylphenidate in Autism Spectrum Disorder: A Long-Term Follow up Naturalistic Study. J Clin Med. 2020;9(8).
196. Lungaro L, Costanzini A, Manza F, Barbalinardo M, Gentili D, Guarino M, et al. Impact of Female Gender in Inflammatory Bowel Diseases: A Narrative Review. Journal of Personalized Medicine. 2023;13(2).
197. Li L, Yao H, Zhang L, Garcia-Argibay M, Du Rietz E, Brikell I, et al. Attention-deficit/hyperactivity disorder is associated with increased risk of cardiovascular diseases: A systematic review and meta-analysis. JCPP Adv. 2023;3(3):e12158.
198. Bang Madsen K, Robakis TK, Liu X, Momen N, Larsson H, Dreier JW, et al. In utero exposure to ADHD medication and long-term offspring outcomes. Molecular psychiatry. 2023;28(4):1739-46.