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ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD) SYMPTOM SEVERITY AND GUT MICROBIOME DYSBIOSIS: AN AETIOLOGICAL PERSPECTIVE ON AN EMERGING RELATIONSHIP

by

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Declaration

I, Katrien van Zyl, declare that this dissertation (in article format) hereby submitted by me for the Magister Articum degree (Clinical Psychology) at the University of the Free State is my own independent work and has not previously been submitted by me to another university/faculty.

Furthermore, I hereby declare that I am aware that the copyright of this dissertation is vested in favour of the University of the Free State.

Katrien van Zyl

14 July 2021

Date

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Abstract

Attention deficit/hyperactivity disorder (ADHD) is the most prevalent neurodevelopmental disorder in the world with a range of adverse outcomes. Collaboration between researchers, healthcare practitioners and families to enhance preventative- and treatment strategies informed by aetiological conceptualisations is key to its management. ADHD presents a complex clinical picture. Genetic heredity and known environmental risk factors do not account for the full spectrum of presenting cases, and many children do not respond to psychopharmaceutical treatments. The role of the gut microbiome in psychiatric disorders has been receiving exponential research interest, radically reconfiguring conceptualisations of mental health. Gut microbiome research beyond microbial analyses is in its infancy, especially with children diagnosed with ADHD. This study is the first focused specifically on the overlapping aetiological factors involved through a clinical psychological lens.

A quantitative dominant mixed methods research (MMR) approach was the most appropriate to address this complex, multifaceted research inquiry comprehensively. Consequently, a total of 197 research participants were selected on the basis of being the biological parent of a child between the age of 6 and 12 who has been formally diagnosed with ADHD in South Africa. Each participant completed the following online surveys: the *ADHD Rating Scale-IV,* the *6-Item Gastrointestinal Severity (GIS) Index* and a *self-compiled biographical questionnaire.* The relationship between symptoms of gut dysbiosis and ADHD symptom severity was explored from a quantitative perspective. A statistically significant correlation was found between *GIS Index total score* and *ADHD Inattention subscale* ($p = 0.026$). Four independent variables were found to make significant contributions *(i.e., maternal prenatal smoking, mode of delivery, breastfeeding, and maternal prenatal obesity)*, although no substantial mediating role by *GIS Index symptoms* were found. The qualitative results elicited parents' observations of the influence of specific factors (e.g., gastrointestinal symptoms, diet, probiotic supplements, worm/parasite treatment) on ADHD symptoms. General concerns regarding children's gut health and the adverse side-effects experienced from psychopharmaceutical treatments were also elicited. This study provides future researchers with a general overview of the overlap between the risk/protective factors involved in ADHD and gut dysbiosis, whilst elucidating the various bidirectional pathways involved in the intricate, emerging relationship between the gut microbiome and mental health.

Key words: ADHD aetiology; gut microbiome dysbiosis; risk/protective factors; South Africa

Literature Review

Introduction and Overview

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterised by a persistent pattern of age-inappropriate levels of hyperactivity, impulsivity, and/or inattentiveness, resulting in clinically significant functional and/or developmental impairments in multiple settings (DSM-V; American Psychiatric Association [APA], 2013). According to the latest version of the Diagnostic and Statistical Manual (DSM-V; APA, 2013), a diagnosis of ADHD can be classified according to three subtypes, namely predominantly hyperactive impulsive, predominantly inattentive, and a combined type, each differing in symptomatology and requiring at least 6 symptoms to be displayed by children for at least 6 months. ADHD is the most frequently diagnosed childhood psychiatric disorder in the world, estimated to affect at least 5% of children globally as well as within the South African context (APA, 2013; Bakare, 2012; Meyer et al., 2004; Polanczyk et al., 2014; Simon et al., 2009; Thomas et al., 2015). According to Polanczyk et al. (2014), the prevalence rates are gradually escalating, with an approximated 21.8% increase in ADHD diagnoses from 2003 to 2007, whilst the Centres for Disease Control and Prevention (2017) have estimated an increase of 43% in the last 15 years.

These statistics inevitably indicate increased pressure on public healthcare and education systems, as well as on the child's family members, especially when taking into consideration the increased risk for adverse outcomes (Barbaresi et al., 2013; Connor et al., 2010; Cussen et al., 2012; Falk et al., 2015; Hoza, 2007; Loe & Feldman, 2007). ADHD has been associated with a range of negative personal, psychosocial, and socioeconomic outcomes, particularly pertaining to educational attainments (Loe & Feldman, 2007), peer and family relationships (Cussen et al., 2012; Hoza, 2007), as well as substance abuse, reckless behaviours and difficulties sustaining employment later in life (Harpin, 2005; Nigg, 2013; Sharma & Couture, 2013).

Children with ADHD appear to experience an array of psychosocial and academic challenges in their formative years. Their scholastic performance is frequently negatively affected with poorer grades in relation to their peers, increasing the risk of repeating grades, truancy, receiving detention, and getting expelled from school, with consequent lower educational and employment attainments (Harpin, 2005; Loe & Feldman, 2007). In addition,

Hoza (2007) reported the adverse influence of an ADHD diagnosis on children's relationships with peers, finding higher rates of interpersonal problems and increased likelihood of rejection and victimisation by their peers. Peer problems may negatively impact the development of a healthy self-esteem and important psychosocial skills such as cooperation, negotiation, and conflict resolution, which are critical for effective social functioning throughout life (Hoza, 2007).

ADHD not only has a significant impact on the child, but also on the child's family (Harpin, 2005; Pheula et al., 2011). Family dysfunction appears higher in families with a child with ADHD, where studies have found increased rates of conflict and lower levels of family cohesion (Harpin, 2005; Pheula et al., 2011). Similarly, Cussen et al. (2012) have found lower family quality of life, less consistent parenting, less parental warmth, and more hostile parenting styles in families with a child diagnosed with ADHD.

Originally seen as a childhood disorder, the lifelong prevalence of ADHD with the cumulative risk for long-term adverse outcomes is now widely acknowledged (Barbaresi et al., 2013; Connor et al., 2010; Cussen et al., 2012; Falk et al., 2015; Hoza, 2007; Loe & Feldman, 2007; Meyer et al., 2004; Nigg, 2013). Adolescents and adults with ADHD frequently encounter problems related to substance abuse, motor vehicle accidents, arrests, suicidality, and health related impairments such as obesity, hypertension, diabetes, and risky sexual behaviours (Harpin, 2005; Nigg, 2013; Sharma & Couture, 2013). Throughout adulthood, ADHD can furthermore increase the risk for inferior job performance, lower socioeconomic status, and occupational and relational instability, overall predicting a lower quality of life for individuals with chronic ADHD (Harpin, 2005).

The risk for adverse outcomes in ADHD can furthermore be enhanced by the range of comorbid disorders which frequently accompany a diagnosis of ADHD, such as oppositional defiant disorder, conduct disorder, major depressive disorder, anxiety disorders, autism spectrum disorder, intellectual disability and developmental disorders related to learning, language, and motor functioning (Akmatov et al., 2019; Bakare, 2012; Caye et al., 2016; Connor et al., 2010; Kessler et al., 2014; Pliszka, 2000). It has been estimated that approximately 65% of individuals with ADHD have at least one comorbid psychiatric disorder, and evidence suggests that "complex" ADHD (i.e., ADHD accompanied by other psychiatric disorders) is the rule rather than the exception (Bierderman et al., 1992; Connor et al., 2010; Seymour & Miller, 2017).

The clinical picture of ADHD is furthermore complicated by the range of physical comorbid conditions related to autoimmune-, allergic-, immunological-, respiratory- and metabolic problems (e.g., atopic eczema, asthma, and allergic rhinitis), which can enhance ADHD symptom severity and the risk for adverse outcomes (Akmatov et al., 2019; Marrs & Flohr, 2016; Miyazaki et al., 2017; Romanos et al., 2011; Verlaet et al., 2014).

Comorbid disorders are widely recognised to modify the nature of the clinical symptom presentations, developmental trajectories, and corresponding adverse outcomes, thus necessitating accommodation of a greater degree of complexity in terms of ADHD management and the consequent enhancement of treatment strategies (Azeredo et al., 2018; Bakare, 2012; Caye et al., 2016; Connor et al., 2010; Kessler et al., 2014; Melegari et al., 2018; Pliszka, 2000; Sharma & Couture, 2013).

The most effective treatment strategies appear to follow a multimodal approach, combining psychotherapeutic interventions with pharmacotherapy and dietary interventions (Abikoff, 2004; Caye et al., 2017). Due to the growing lack of access to mental health resources in Africa, a multimodal integrative strategy is often not feasible for families with a low socio-economic status (Bakare, 2012; Meyer et al., 2004). In South African public healthcare, pharmacotherapy, in the form of stimulant medication (i.e., methylphenidate), is the most frequently prescribed and readily available treatment option due to its ease of administration and relatively successful response rates (Schoeman & De Klerk, 2017).

Even though stimulant medication has proven to have short-term efficacy in the alleviation of ADHD symptoms, research has found that it is still unable to improve symptoms in 20-35% of presenting cases (Caye et al., 2017; Childress & Sallee, 2014; Greenhall et al., 2001). Furthermore, stimulant medication is often avoided due to the range of associated risks and negative side effects, such as insomnia, loss of appetite, weight-loss, nausea, muscle tension, headaches, listless appearance, and cardiovascular problems in some cases (Hodgson et al., 2014; Purdie et al., 2002). As a result, drug holidays are frequently recommended and there is a growing need for alternative treatment options around the world (Greenhill et al., 2000; Hodgson et al., 2014; Vogel, 2013).

Despite many barriers to treatment, the majority of children respond well to stimulant medication, which has been found to enhance the functioning of key neurotransmitters involved in ADHD (i.e., dopamine and norepinephrine), consequently informing the general

neurological conceptualisation of ADHD pathophysiology (Sharma & Couture, 2013). However, this inevitably beckons the question as to why a large portion of children diagnosed with the same disorder shows no response to the same medication (Caye et al., 2017; Childress & Sallee, 2014; Greenhall et al., 2001).

Researchers have attributed the observed heterogeneity in terms of response to pharmaceutical treatments and symptom presentation, in part, to the heterogeneity of aetiological factors, and many believe that ADHD treatment and prevention strategies can be enhanced through more rigorous ADHD aetiological research (Cortese & Coghill, 2018; Nigg, 2012; Sciberras et al., 2017; Sharma & Couture, 2013; Silva et al., 2014; Tarver et al., 2014; Thapar et al., 2012; van Dyk et al., 2014). This study therefore aims to provide a unique perspective on ADHD aetiology within the South African context where there still appears to be a general shortage of research in this field.

ADHD Aetiology

ADHD is categorised as a neurodevelopmental disorder in the 5th edition of the Diagnostic and Statistical Manual (DSM-5), indicating developmental deficits in the central nervous system (CNS) that typically have an early onset (APA, 2013). Genetic factors are generally accepted to play a significant aetiological role in most neurodevelopmental disorders (Cenit et al., 2017; Nigg, 2012; Pham, 2015; Zayat & Neale, 2020). Evidence gathered from twin and family studies indicate that approximately 60%-90% of ADHD cases can be accounted for by genetic heredity (Faraone et al., 2005; Waldman & Gizer, 2006; Zayats & Neale, 2020). This results in an estimated 10-40% of the aetiological variance being accounted for by environmental risk factors (Banerjee et al., 2007; Hamza et al., 2017; Mirkovic et al., 2020).

Systematic literature reviews agree on the following prominent environmental factors in ADHD: maternal substance use (i.e. alcohol and tobacco); maternal prenatal stress; prematurity and low birth weight; exposure to toxins (i.e. heavy metals, organophosphates, polychlorinated biphenyls); early malnutrition (i.e. lack of breastfeeding and unhealthy childhood diet); and psychosocial risk factors (e.g., early childhood deprivation, maternal hostility, parent-child conflict) (Cortese & Coghill, 2018; Nigg, 2012; Sciberras et al., 2017; Sharma & Couture, 2013; Silva et al., 2014; Tarver et al., 2014; Thapar et al., 2012; van Dyk et al., 2014).

This broad range of environmental risk factors, in combination with the significant genetic aetiological foundation of ADHD, necessitates the consideration of complex gene-environment interactions (Mill & Petronis, 2008). In recent years, ADHD aetiology is increasingly being viewed through an epigenetic lens, which holds that genetic heritage is not necessarily deterministic, instead it can be viewed as a predisposition which can be precipitated by certain environmental risk factors (Ficks & Irwin, 2009; Hamza et al., 2017; Mill & Petronis, 2008; Mirkovic et al., 2020; Schuch et al., 2015).

Epigenetic research holds important implications for the conceptualisation of ADHD aetiology in that it shifts the dominant scientific narrative from genetic heredity towards a more integrated, systematic investigation of interactions between genes and the various environmental risk factors involved in this disorder (Hamza et al., 2017). These factors are evidently multidimensional and broad in scope, creating a complex aetiological foundation, which may explain the high degree of clinical heterogeneity observed in both symptomatic presentations and response to treatment strategies.

ADHD appears to be one of the most well researched disorders in the twenty-first century. However, even though significant progress has been made towards understanding the various pathogenic mechanisms involved in ADHD, there is consensus amongst the scientific community that this disorder continues to portray a highly heterogenous clinical picture and that further investigation is required to establish a rigorous aetiological framework from which prevention, diagnostic and treatment strategies can be enhanced (Banaschewski et al., 2017; Cenit et al., 2017; Froehlich et al., 2011; Nigg, 2012; Sciberras et al., 2017; Verlaet et al., 2014).

Neurodevelopment and the Gut Microbiome

Clinical heterogeneity may be explained, in part, by the presence of mediating factors in the pathways between genetic predispositions, environmental risk factors and the development of ADHD symptoms (Banaschewski et al., 2017; Casas et al., 2019). Mediating factors refer to the potential mechanisms by which an independent variable can produce changes in a dependent variable (Cenit et al., 2017). In this regard, it has been hypothesized that the gut microbiome may play an important mediating role between several identified environmental risk factors and the development of ADHD (Akram, 2017; Cenit et al., 2017).

The gut microbiome refers to the compilation of roughly 100 trillion microorganisms in an individual's intestinal lining and consists of a broad range of microbial cells (Strandwitz,

2018). These cells collectively outnumber the total number of human cells in the body by at least 10 to 1, and genetically outnumber the human genome by 100 to 1 (Allen, 2017; Clapp et al., 2017; Ghaisas et al., 2015). Our conceptualisation of what it means to be "human" has thus been radically reconfigured in what has come to be known as nothing less than a momentous paradigm shift in neuro- and behavioural science (Allen et al., 2017). According to Allen et al. (2017; p.2), "the study of how microbes within the body can interact with the human brain and behaviours can offer a more complete understanding of human psychology". The gut-brain connection has evidently become an area in which the field of psychology can make a valuable contribution.

Researchers are increasingly discovering that the gut microbiome has a significant impact not only on our physical health, but also on our behaviour, mood, stress responses and cognition, which presents important implications for mental health (Allen et al., 2017; Dinan & Cryan, 2017; Sherwin et al., 2016). The healthy integrity and proper functioning of the gut microbiome depend on the intricate balance and diversity of benevolent microorganisms in the intestinal tract, known as *eubiosis*, a state in which the inner ecological environment is in homeostasis (Allen et al., 2017; Cenit et al., 2017; Deans, 2016; Foster & Zhou, 2015; Noble et al., 2017; Sharon et al., 2016). A disturbance in this balance is referred to as gut (microbiome) dysbiosis, which has been associated with a range of mental disorders, such as major depressive disorder, bipolar disorder, generalised anxiety disorder, schizophrenia, and autism spectrum disorder (Capuco et al., 2020; Chrobak et al., 2016; Dash et al., 2015; Dawson et al., 2016; Deans, 2016; Dillan & Cryan, 2017; Golofast & Vales, 2020; Kelly et al., 2020; Mulle et al., 2013; Rogers et al., 2016; Sharon et al., 2016).

In recent years, significant associations between gut dysbiosis and neurodevelopmental disorders are being found (Forssber, 2019; Lacorte et al., 2019; Warner, 2018). With regards to research within the paediatric population, children on the autism spectrum have received the most interdisciplinary investigation to date, however, researchers are increasingly implicating gut dysbiosis in the underlying pathophysiology of ADHD (Boonchooduang et al., 2020.; Bull-Larson & Mohajeri, 2019; Casas et al., 2019; Dam et al., 2019; Lacorte et al., 2019; Mathee et al., 2020; Ming et al., 2018; Prehn-Kristensen et al., 2018; Sandgren & Brummer, 2018; Sukmajaya et al., 2021; Tognini, 2017; Wang et al., 2020). In support of this hypothesis, several studies have found differences in gut microbiome compositions between ADHD patients and control groups (Jiang et al., 2018; Lacorte et al., 2019; Prehn-Kristensen et al.,

2018; Szopinska-Tokov et al., 2020; Wan et al., 2020), whilst chronic gut dysbiosis related symptoms, such as constipation, flatulence, and abdominal pain, have been found in a significant proportion of children diagnosed with ADHD (McKeown et al., 2013; Ming et al., 2018).

Gut dysbiosis can manifest in a range of gastrointestinal symptoms, although it easily remains undetected due to its chronic, heterogeneous nature and often subtle presentation, especially in the younger population (Bischoff, 2011; Ming et al., 2018). Gut dysbiosis can also present itself in a more indirect manner through an array of associated physiological conditions, such as atopic eczema, asthma, allergies, and obesity, all of which are frequently comorbid with ADHD (Akmatov et al., 2019; Belkaid & Hand, 2014; Chen et al., 2013; Hak et al., 2013; Johnson & Ownby, 2016; Leffa et al., 2018; Marrs & Flohr, 2016; Mitchell et al., 2014; Miyazaki et al., 2017; Zhou et al., 2017).

There is thus ample evidence to suspect that the gut microbiome can play an important role in the relationship between ADHD and environmental risk factors. However, even though there has been a surge of gut-microbiome-brain related research in recent years, most studies have been conducted within a microbiological framework which inherently lacks the humanistic component with a focus on the interrelated nature of the various factors influencing this complex, multidimensional disorder. Hence, there appears to be an evident need for more integrated research which could provide an aetiological overview of ADHD from a clinical psychological perspective.

The Gut-Brain Axis

The gut microbiome and the brain are constantly engaged in bidirectional communication through what has come to be known as the microbiome-gut-brain axis (MGBA), or simply the gut-brain axis (GBA) (see figure 1) (Akram, 2017; Cenit et al., 2017; Checa-Ros, 2021; Dinan & Cryan, 2017; Ming et al., 2018; Muhammad et al., 2020). The brain constantly sends and receives signals from the gut, and vice versa, via several mechanisms, which have not yet been fully elucidated, but include nerve-, neuroendocrine-, immune-, and metabolic pathways (Akram, 2017; Checa-Ros, 2021; Dinan & Cryan, 2017; Sharon et al., 2016; Tognini, 2017). According to Breit et al. (2018), the GBA is responsible for maintaining physiological homeostasis and connecting the emotional and cognitive areas of the brain with peripheral intestinal functions, such as enteroendocrine signalling, intestinal permeability, and immune activation.

The gut-brain axis predominantly operates through the vagus nerve, which connects the gastrointestinal tract and brain in a bidirectional fashion and has proven to play a substantial role in anxiety and mood regulation (Breit et al., 2018). The vagus nerve forms an integral part of the enteric nervous system (ENS), a large, intricate network of nerves in the gut lining which is directly connected to the central nervous system (CNS) (Cenit et al., 2017; Sharon et al., 2016; Tognini, 2017). This complex communication system functions primarily through neurotransmitters, which forms the foundation of neuropsychology, a field of psychology that is now expanding to encompass a firm theoretical understanding of the integral role of the gut microbiome (Allen et al., 2017; Sherwin et al., 2016; Dean, 2016).

The ENS contains more neurons than the spinal column and produces more than 30 neurotransmitters in collaboration with the gut microbiome (Breit et al., 2018). Studies have indicated that the gut microbiome can play a vital role in the synthesis of key neurotransmitters involved in ADHD, namely dopamine, norepinephrine, and serotonin, with as much as 90% of the body's serotonin produced in the gut (Gershon & Tack, 2007; Mathee et al., 2020; Petra et al., 2015; Reigstad et al., 2015; Strandwitz, 2018). These neurotransmitters are responsible for modulating cognitive control of motivation, behaviour and reward perception in the prefrontal cortex and striatum, which play a central role in the pathophysiology of ADHD (Akram, 2017; Cenit et al., 2017; Lacorte et al., 2019; Mathee et al., 2020; Ming et al., 2018; Prehn-Kristensen et al., 2018; Wang et al., 2020).

Through a neurodevelopmental lens, the gut microbiome furthermore plays a central role in neurogenerative processes, which include myelination, microglia development and the formation of the blood-brain barrier (BBB) (Sharon et al., 2016; Sherwin et al., 2016). In addition, the gut microbiome can regulate immunity, inflammation, hormonal activity, and the hypothalamic-pituitary-adrenal (HPA) axis, as well as the production of brain-derived neurotropic factor (BDNF), all of which are known to impact neurodevelopment (Cenit et al., 2017; Dinan & Cryan, 2017; Kelly, 2016; Sherwin et al., 2016).

Researchers are therefore increasingly discovering that the gut microbiome can significantly influence neurodevelopment and neurocognitive functioning via the gut-brain axis, although there is consensus that more interdisciplinary research is required to elucidate the mechanisms through which this takes place with a focus on the specific aetiological factors involved (Akram, 2017; Cenit et al., 2017; Dinan & Cryan, 2017; Sharon et al., 2016; Tognini, 2017).

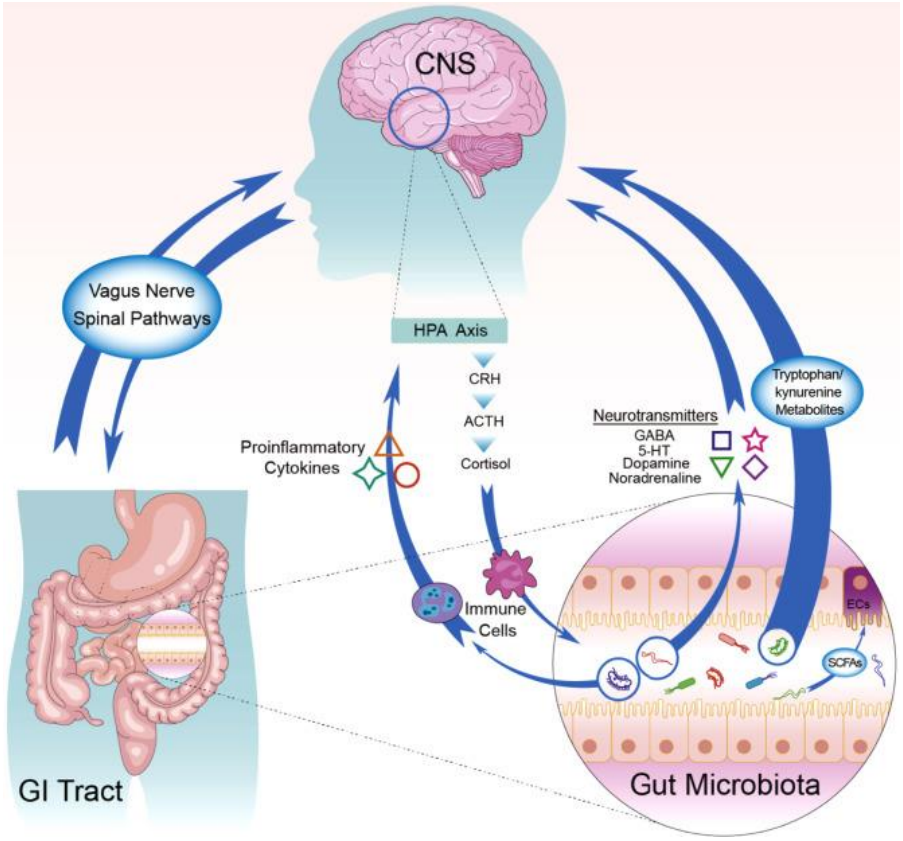

Figure 1: The Gut-Brain Axis (Muhammad et al., 2020)

ADHD and Gut Dysbiosis: Aetiological Overlap

Neurodevelopment is a fragile, intricate process, which can be influenced by a range of factors that integrate genetic and environmental cues (Sharon et al., 2016). According to Kelly et al. (2016), epigenetic factors involved in shaping behaviours could arise as a consequence of host-microbiome interactions. In this regard, it appears as though most ADHD aetiological studies have solely focused on the human genome (i.e., DNA profile), overlooking the pivotal role played by the second, vastly larger, genome contained in the gut microbiome (Allen, 2017; Clapp et al., 2017; Ghaisas et al., 2015).

ADHD research has expanded exponentially in recent years as the prevalence and impact of this disorder continues to grow, and researchers are increasingly implicating the gut microbiome as an important pathophysiological mechanism in ADHD (Bull-Larson & Mohajeri, 2019; Casas et al., 2019; Lacorte et al., 2019; Mathee et al., 2020; Ming et al., 2018; Prehn-Kristensen et al., 2018; Sandgren & Brummer, 2018; Tognini, 2017; Wang et al., 2020). One consistent finding across several systematic research reviews indicates that exposure to the known environmental risk factors (i.e. maternal prenatal substance use and stress;

prematurity and low birth weight; exposure to toxins; early malnutrition; and psychosocial adversity) occurs early in life during the neonatal and postnatal period when infants are most susceptible to neurodevelopmental alterations (Casas et al., 2019; Cortese & Coghill, 2018; Nigg, 2012; Sciberras et al., 2017; Sharma & Couture, 2013; Tarver et al., 2014; Thapar et al., 2012; van Dyk et al., 2014).

It is well known that neurodevelopment is significantly affected by maternal health factors such as maternal immunity, nutrition, metabolism, obesity, substance use and stress levels, which formed the theoretical foundation for related aetiological research (Nigg, 2012; Sciberras et al., 2017; Sharma & Couture, 2013; Tarver et al., 2014; Thapar et al., 2012). Even though there is still a lack of understanding related to the specific pathophysiological interactions involved, it has been hypothesised that these factors alter the mother's microbiome composition, which then significantly impacts the formation of the infant's gut microbiome and thus neurodevelopmental trajectory (Rogers et al., 2016; Singh & Mittal, 2019).

Originally it has been accepted that infants are born with a sterile gut environment, however, recent studies have discovered that the infant's gut microbiome already starts to develop in utero through contact with the mother's microbiome (Collado et al., 2016; Nagpal & Yamashiro, 2017; Sharon et al., 2016; Singh & Mittal, 2019; Walker et al., 2017). It has consequently been proposed that maternal microbiome dysbiosis can alter normal foetal microbial and neurological development before, during and after birth (Cenit et al., 2017; Rogers et al., 2016; Singh & Mittal, 2019; Walker et al., 2017). The infant thus not only inherits the mother's DNA, but also her unique microbiome genetic profile. Postnatally, an infant's microbiome is established through breastmilk, diet and contact with the environment during the first 2-3 years of life, maturing parallel to the critical period of neurodevelopment (Casas et al., 2019; Cenit et al., 2017; Cerdo et al., 2016; Lu & Claud, 2018; Sharon et al., 2016). The first few years of an infant's life is thus vital for both brain and gut microbiome development.

Even though associations between gut dysbiosis and ADHD aetiological factors have been suggested (Cenit et al., 2017; Mathee et al., 2020; Prehn-Kristensen et al., 2018), no studies to date have focused specifically on the interactions between the relevant factors and there appears to be an overall shortage of ADHD research within the South African context (Bakare, 2012). Cenit et al. (2017), proposed several important similarities between environmental risk factors related to the development of ADHD and gut dysbiosis (see figure 2), which will be explored and expanded upon in the following section.

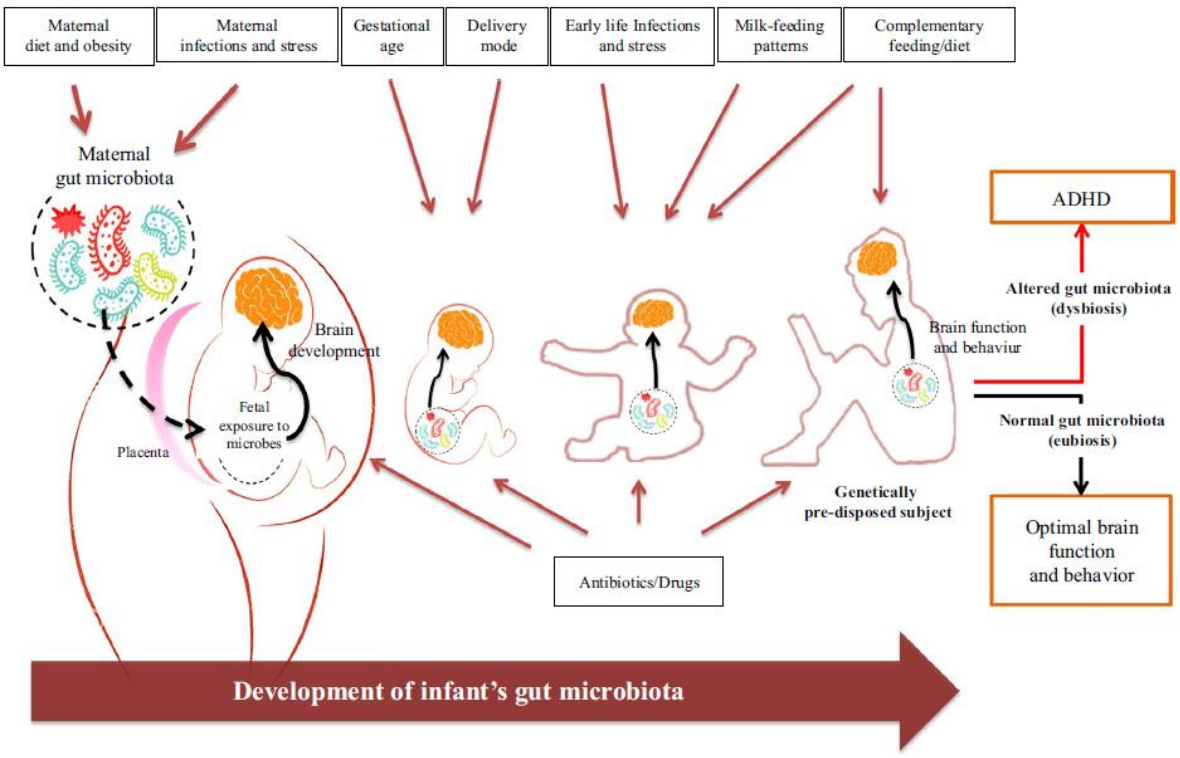

Figure 2: Schematic representation of environmental factors (Cenit et al., 2017; p.3)

Mode of Delivery, Gestation Age and Birthweight

ADHD aetiological research reviews concur on the risks posed by premature birth, and consequent low birth weight, where preterm infants appear to have a greater risk of developing ADHD and are described to have more severe ADHD symptoms in comparison to control groups (Cortese & Coghill, 2018; Frantz, 2017; Lim et al., 2018; Nigg, 2012; Pettersson et al., 2015; Sciberras, 2017; Sharma, 2013; Tarver, 2014; Thapar, 2012). This may be attributed to the neurodevelopment in utero being interrupted by a premature birth in combination with increased risk for breastfeeding difficulties, maternal separation, and infections in the postnatal period (Cong et al., 2015; Groer et al., 2015; Lim et al., 2018).

Given the novel awareness that a neonate's gut microbiome already starts developing in utero, it would be logical to conclude that this process was also slightly halted by a preterm birth, which may be why differences are being found in infant gut microbial compositions depending on the duration of pregnancy (i.e., gestation age) (Chernikova et al., 2018; Collado et al., 2016; Cong et al., 2015; Lim et al., 2018; Nagpal & Yamashiro, 2017; Pettersson et al., 2015; Sharon et al., 2016). These differences may also be explained, in part, by differences in

the maternal microbial profiles as researchers are finding associations between maternal microbiome dysbiosis and preterm births (Cao et al., 2014; Cong et al., 2015; Satude et al., 2018). These new findings present interesting possible interactions between gestation age, maternal microbiome dysbiosis, in utero gut microbiome formation, and ADHD aetiology.

In terms of mode of delivery, it has been established that an infant's gut microbiome is seeded/colonised to a large extent through birth (Axelsson et al., 2018; Lemas et al., 2016; Nagpal & Yamashiro, 2017). During a natural (vaginal) delivery, the infant is exposed to a diversity of microbes in the birth canal, whereas with a Caesarean-section (C-section) delivery, the infant is only exposed to microbes on the mother's skin and in the immediate environment directly after birth (Cenit et al., 2017; Cong et al., 2015; Lemas et al., 2016; Nagpal & Yamashiro, 2017).

According to literature reviews by Nagpal and Yamashiro (2017) and Hoang et al. (2020), C-section births may result in decreased microbial diversity, which may explain why this mode of delivery seems to predispose individuals to developing related health problems later in life. C-section births have also been implicated in ADHD aetiology (Axelsson et al., 2018; Cenit et al., 2017; Talge et al., 2016). However, conflicting findings still abound in research with aetiological associations also found between ADHD and natural births, which could possibly be attributable to maternal health problems and birth complications (Wagner et al., 2009). Consequently, there is an expressed need for further studies to draw more definitive conclusions about the role of delivery mode in ADHD aetiology (Axelsson et al., 2018; Cenit et al., 2017; Talge et al., 2016).

Maternal Substance Use, Environmental Toxins and Antibiotics

The prenatal environment evidently plays a significant role in neurodevelopment. ADHD aetiological research has proven that prenatal maternal substance use, particularly tobacco and alcohol, significantly increases the risk for ADHD (Cortese & Coghill, 2018; Eilertsen et al., 2017; Han et al., 2015; He et al., 2020; Nigg, 2012; Sciberras, 2017; Sharma, 2013; Tarver, 2014; Thakur et al., 2012; Thapar, 2012). Thakur et al. (2012) found a dose dependant correlation between the average amount of cigarettes smoked per day and neurocognitive functioning deficits. Similarly, Eilertsen et al. (2017) found a dose-reliant causal relationship between alcohol consumption and the development of ADHD. Capurso and Lahner (2017) demonstrated that prolonged, excessive tobacco and alcohol consumption has a neurotoxic effect and can cause disturbances in the mother's gut microbiome, which may

present one pathway through which the neonate's neurodevelopment may be impacted in this respect.

Other environmental toxins, in the form of heavy metals (e.g., lead) and pesticides (e.g., organophosphates & polychlorinated biphenyls), have proven to be significant risk factors in the aetiology of ADHD (Cortese & Coghill, 2018; Nigg, 2012; Sciberras et al., 2017; Sharma & Couture, 2013; Tarver et al., 2014; Thapar et al., 2012; van Dyk et al., 2014). Tu et al. (2020) explained that early exposure to these neurotoxic chemicals can significantly disrupt the integrity of the gut microbiome and emphasised the important role played by the commensal gut microbes in ameliorating the impact through the biotransformation of toxins into less harmful metabolic substrates.

Tu et al. (2020) also classified antibiotics, particularly broad-spectrum antibiotics, amongst the harmful neurotoxic chemicals with dire consequences for the health of the gut microbiome. Antibiotics have proven to be effective against a range of pathogenic bacterial species and it is therefore not surprising that it is amongst the most frequently prescribed medication for children in the world (Neuman et al., 2018). The surge in microbiome related research has revealed that even brief antibiotic exposure in childhood can significantly alter microbial compositions, often resulting in gut dysbiosis with long-term health implications, increasing the risk for asthma, allergic rhinitis, atopic dermatitis, celiac disease, and obesity (Aversa et al., 2020; Cenit et al., 2017; Neuman et al., 2018; Sharon et al., 2017; Tu et al., 2020).

In terms of the potential contribution of early antibiotics exposure in the aetiological framework of ADHD, there appears to be a need for more empirical evidence. Axelsson et al. (2018) investigated the association between early antibiotic treatment and the development of ADHD over the course of 15 years, although inconclusive results were obtained. Slykerman et al. (2019), on the other hand, also conducted a longitudinal study and found that children who had received antibiotics in the first year of life had a significantly increased risk for ADHD symptoms, anxiety, and emotional problems with lower overall cognitive-, verbal-, and executive functioning abilities. Similarly, Aversa et al. (2020) found that exposure to antibiotics during the first 2 years of life increased the risk for ADHD development.

Antibiotic treatments have varying degrees of neurotoxic effects on the enteric nervous system and the central nervous system, altering neurotransmitter signalling and impacting

neurodevelopment (Champagne-Jorgensen et al., 2018). Antibiotics furthermore destroy benevolent commensal bacteria in the gut, with minimal impact to the mycobiome (i.e., the fungal community in the gut), allowing fungal pathogens, such as *Candida albicans*, to grow out of balance and cause gut dysbiosis in the form of a yeast/fungal infection (i.e., *Candidiasis*), which has a range of endotoxic implications (Dinsmoor et al., 2005; Garcia-Gamboa et al., 2021; Rucklidge, 2013). In this regard, a 3-year case study by Rucklidge (2013) found that ADHD and mood symptoms improved following antifungal and probiotic treatment of *Candidiasis*, outlining the importance of gut health when treating psychiatric disorders.

Maternal Health and Obesity

The health of a neonate's mother during pregnancy also appears to have a significant impact on neurodevelopment. Mothers who struggle with health concerns are more likely to use antibiotics intrapartum, which has been shown to enhance their risk for developing a fungal/yeast infection and gut dysbiosis (Dinsmoor et al., 2005; Lemas et al., 2016). Furthermore, mothers who opt for C-sections often receive courses of antibiotics in the perinatal period, which may furthermore impact the infant's gut microbiome development through breastfeeding (Lemas et al., 2016; Nagpal & Yamashiro, 2017).

Mothers who struggle with gut dysbiosis are also more likely to be overweight during pregnancy (Garcia-Gamboa, 2021). Food preferences are affected by the gut microbial composition, which in turn is shaped by diet (Dinsmoor et al., 2005). For example, pathogenic yeast such as *Candida albicans* thrive on carbohydrates and release a specific toxin which induces cravings for foods rich in carbohydrates such as sugar and bread (Ruckland, 2013). In general, the modern Western diet typically consists of carbohydrate-rich processed foods and unhealthy fats, which naturally promotes gut dysbiosis and excessive weight-gain (Edwards et al., 2017).

Maternal obesity has been found to impact neurological development and to increase the risk of developing ADHD (Bazar et al., 2006; Cerdo et al., 2016; Li et al., 2020; Rivera et al., 2015; Rodriguez et al., 2007). Cerdo et al. (2016) found that mothers who struggle with obesity are more likely to have insulin resistance and chronic systemic inflammation, which may be due to pathogenic alterations in their gut microbial composition as gut microbes play a key role in food preferences and moderating inflammation (Gupta et al., 2020). In a recent study by Gustafsson et al. (2020) it was demonstrated that maternal prenatal inflammation was directly related to increased risk for ADHD symptoms in their offspring.

Other manifestations of prenatal maternal health concerns, such as urinary tract infections, diabetes, and psoriasis, resulting in immune activation and gut microbiome alterations, have also been associated with increased risk for the development of ADHD (Ginsberg et al., 2018; Nielsen et al., 2021). These findings may suggest associations between ADHD aetiology and maternal health and gut microbiome dysbiosis before, during and after birth.

Immunity and Inflammation

Like many mothers, children with ADHD also frequently struggle with more health problems in relation to their peers, supporting the hypothesis that ADHD symptoms can be related to immune dysregulation (Hak et al., 2013; Johnson & Ownby, 2016; Verlaet et al., 2014; Zhou et al., 2017). The gut microbiome has a strong modulating effect on the immune system throughout life and plays a particularly important role in its development during infancy (Sanidad et al., 2020). The immune system, in turn, regulates inflammation, which is the body's natural response to pathogens, infections, injuries and toxins (Xue et al., 2020). If the immune system and consequent inflammatory response is chronically activated, however, it can have a range of negative health outcomes (Anand et al., 2017; Akmatov et al., 2019; Leffa et al., 2018).

According to Sanidad et al. (2020), perturbations in the neonatal gut microbiome can increase the risk of inappropriate activation of immune cells and chronic systemic inflammation, resulting in abnormally increased levels of pro-inflammatory cytokines, which has been discovered in a significant portion of children with ADHD symptoms (Anand et al., 2017; Leffa et al., 2018; Marrs & Flohr, 2016; Mitchell et al., 2014; Romanos et al., 2011; Verlaet et al, 2014; Zhou et al., 2017). This may explain why both gut dysbiosis and ADHD are frequently found to be comorbid with inflammatory conditions such as asthma, allergies, and eczema (Akmatov et al., 2019; Belkaid & Hand, 2014; Chen et al., 2013; Hak et al., 2013; Johnson & Ownby, 2016; Leffa et al., 2018; Mitchell et al., 2014; Miyazaki et al., 2017; Zhou et al., 2017).

Chronic systemic inflammation can result in the deterioration of the blood-brain barrier (BBB), which can allow pathogens and neurotoxic chemicals to affect the central nervous system (CNS) (Anand et al., 2017; Donev & Thome, 2010; Maynard et al., 2012; Ming et al., 2018). Neuroinflammation can influence the evaluation of sensory stimulation and the regulation of prefrontal cortex responses, including reaction time and working memory, and

may therefore interfere with the neurodevelopmental and cognitive functional deficits observed in ADHD (Anand et al., 2017; Donev & Thome, 2010; Zhou et al., 2017).

Furthermore, when the gut microbiome is in a state of dysbiosis there may be a disproportionate amount of pro-inflammatory microbes present in the gut, increased oxidative stress, and intestinal permeability may be increased, resulting in varying degrees of what has been termed "leaky gut syndrome" (Camilleri, 2019; Donev & Thome, 2010; Leffa et al., 2018). A "leaky gut", or hyperpermeable gut lining, allows toxins, pathogens, and partially digested food particles to seep into the bloodstream, activating the immune system and creating a chronic inflammatory response and increase in histamine levels, which often translates in allergic reactions and conditions such as asthma, eczema, rhinitis, sinusitis, and food intolerances, all of which are frequently co-morbid with ADHD (Camilleri, 2019; Feng et al., 2012; Hill et al., 2016; Leffa et al., 2018; Prince et al., 2015).

Early Life Nutrition and Childhood Diet

In terms of the contribution of early life nutrition in ADHD aetiology, breastfeeding has been found to play a significantly protective role in the development of ADHD along with a range of other psychiatric disorders (Lawrence, 2014; Mimouni-Bloch et al., 2013; Park et al., 2014; Stadler et al., 2016; Tseng et al., 2018). According to Wang et al. (2020), the specific mechanisms involved have, however, not yet been fully elucidated. Cenit et al. (2017) proposed that the gut microbiome should be considered as one of the crucial factors mediating the relationship between breastfeeding and ADHD. In this regard, it has been firmly established that the infant's gut microbiome is formed predominantly through the ingestion of breastmilk (Cerdo et al., 2016; Lemas et al., 2016; Pannaraj et al., 2017).

Within the field of ADHD aetiological research, the relationship between ADHD and general childhood diet has, however, not been as apparent in research findings. The influence of dietary factors on ADHD has been thoroughly investigated since Benjamin Feingold in 1973 first proposed that ADHD symptoms are linked to the ingestion of certain foods (e.g., processed sugar) and food additives (e.g., artificial food colourants and flavourants) (Stevens et al., 2011). This hypothesis has been the subject of much controversy within the ADHD research community as conflicting findings still abound in literature (Rytter et al., 2015; Lange, 2017; Stevens et al., 2011).

A recent meta-analysis and systematic literature review of ADHD and dietary patterns

(Del-Ponte et al., 2019) demonstrated that a diet high in saturated fat, preservatives, artificial food colourants and refined sugar can increase the risk of ADHD, while a healthy diet characterized by the high consumption of fruits and vegetables could act as a protective factor. Similarly, it has been found that addressing certain nutritional deficiencies (e.g., omega-3, magnesium, zinc, & iron), can decrease ADHD symptoms in children (Arnold et al., 2000; El-Bakry et al., 2019; Elbaz et al., 2017; Richardson, 2006). Even though many studies have found associations between ADHD, early life nutrition and childhood diet (Kanarek, 2011; Millichap & Yee, 2012; Pelsser et al., 2017; Stevens et al., 2011), several others have obtained inconclusive results (Cagigal et al., 2019; Kim & Chang, 2011; Nigg & Holton, 2014).

It is logical to suspect that these incongruencies may be due, in part, to the involvement of key mediating factors. Cenit et al. (2017) proposed that the gut microbiome has long been overlooked as an essential mechanistic link between diet and ADHD symptoms. Similarly, Wang et al. (2020) suggested important associations between diet, the gut microbiome and susceptibility to ADHD. Diet may thus influence ADHD by acting directly on the enteric nervous system and indirectly by affecting the gut microbiome (Dam et al., 2019).

It has been established that the gut microbiome composition is significantly influenced by dietary factors (i.e., healthy fibre in fruit and vegetables) and plays an essential role in food digestion, nutrient- absorption and synthesis, as well as protection against toxins and chemicals (Deans, 2016; Kan et al., 2018; Mathee et al., 2020). Gut dysbiosis may therefore result in multiple nutritional deficiencies and increased sensitivity to neurotoxins in food (i.e., artificial colourants and flavourants), which may explain why some children are more susceptible to dietary factors and nutritional interventions than others (Cenit et al., 2017).

In further support of this hypothesis, the proven efficacy of omega-3 supplementation in ADHD treatment (Richardson, 2006) needs to be considered in relation to its impact on the gut microbiome. According to Constantini et al. (2017), omega-3 can exert a positive influence on commensal microbiota composition and increase the production of anti-inflammatory compounds (i.e., short-chain fatty acids) in the gut microbiome. Similarly, Noriega et al. (2016) explain that some of the health benefits of omega-3 may be due to the benevolent impact it has on the gut microbiome (i.e., increases in butyrate producing bacteria). The observed omega-3 treatment efficacy in ADHD may therefore be due, in part, to its positive effect on the gut microbiome composition in children.

Prenatal Maternal Stress and Early Life Stressors

ADHD aetiological research has indicated associations between high prenatal maternal stress and the development of ADHD as well as increased symptom severity (Grizenko et al., 2015; Okano et al., 2018; Rodriguez & Bohlin, 2005; Ronald et al., 2011; Sciberras, 2017). The pathways through which maternal stress influences neurodevelopment remain undetermined, however, it has been reported that prolonged stress during pregnancy disrupts maternal microbial composition and may therefore result in maternal microbiome dysbiosis (Edwards et al., 2017; Glover et al., 2018; Jasarevic, 2015; Rakers et al., 2017; van den Bergh et al., 2017). Maternal microbiome dysbiosis in the gut and reproductive system will lead to modifications in the neonate's microbiome development in utero and through microbial transmissions during birth (Edwards et al., 2017; Glover et al., 2018; Jasarevic, 2015; Singh & Mittal, 2019). The infant may thus be born with gut dysbiosis which may then consequently alter normal neurodevelopmental trajectories (Cong et al., 2015; Glover et al., 2018; Osadchiy et al., 2018; Rea et al., 2016).

Stress may impact neurodevelopment and the health of the microbiome in several ways. Firstly, stress hormones can have a detrimental effect on the integrity of the intestinal barrier and has been associated with increased gut permeability, often resulting in stress-induced "leaky gut syndrome" and consequent neuroinflammation (Kelly et al., 2015; Rea et al., 2016). Secondly, exposure to early stressors can lead to intestinal oxidative stress, which may disrupt the process of healthy gut microbiome development (Cong et al., 2015; Rea et al., 2016). Thirdly, prolonged stress can cause disturbances in the hypothalamic-pituitary-adrenal (HPA) axis, which plays key roles in the gut microbiome, endocrine, and immune system (Rea et al., 2016; Verlaet et al., 2014).

Conversely, commensal gut microbes are critical for the development of normative stress responses as they influence the amount of cortisol the body releases in response to stress via the HPA axis, which regulates the body's stress hormones (Vogel et al., 2020). Interestingly, both Isaksson (2014) and Liang et al. (2011) found significant differences in HPA axis functioning between ADHD children and control groups. It could thus be hypothesised that these differences may be due, in part, to differences in gut microbiome compositions.

In light of the emerging bidirectional relationship between stress and the gut microbiome (figure 3), there has been a surge of research interest in the role of the gut microbiome in an individual's ability to respond to adverse psychosocial circumstances (i.e.,

"stress resilience"), with researchers increasingly suggesting that a healthy gut microbiome may act as a long overlooked protective factor (Foster et al., 2017; Rakers et al., 2017; Rosin et al., 2020; van den Bergh et al., 2017). This has important implications within the South African context where many children are increasingly being exposed to adverse psychosocial conditions (Manyema & Richter, 2019).

Early life stressors, in the form of, for example, mother-infant separation, high family conflict, abuse, and neglect, are generally accepted to have detrimental implications for neurodevelopment and have been proven to enhance the risk for developing ADHD (Humphreys et al., 2018; Manyema & Richter, 2019; Nigg et al., 2020). Researchers have done much to elucidate the specific multidimensional pathways involved in the association between neurodevelopmental disorders and stress and have begun to increasingly implicate the gut microbiome as an important mediating factor in this relationship (Cong et al., 2015; Foster et al., 2017; Rakers et al., 2017; Rosin et al., 2020; van den Bergh et al., 2017; Vogel et al., 2020).

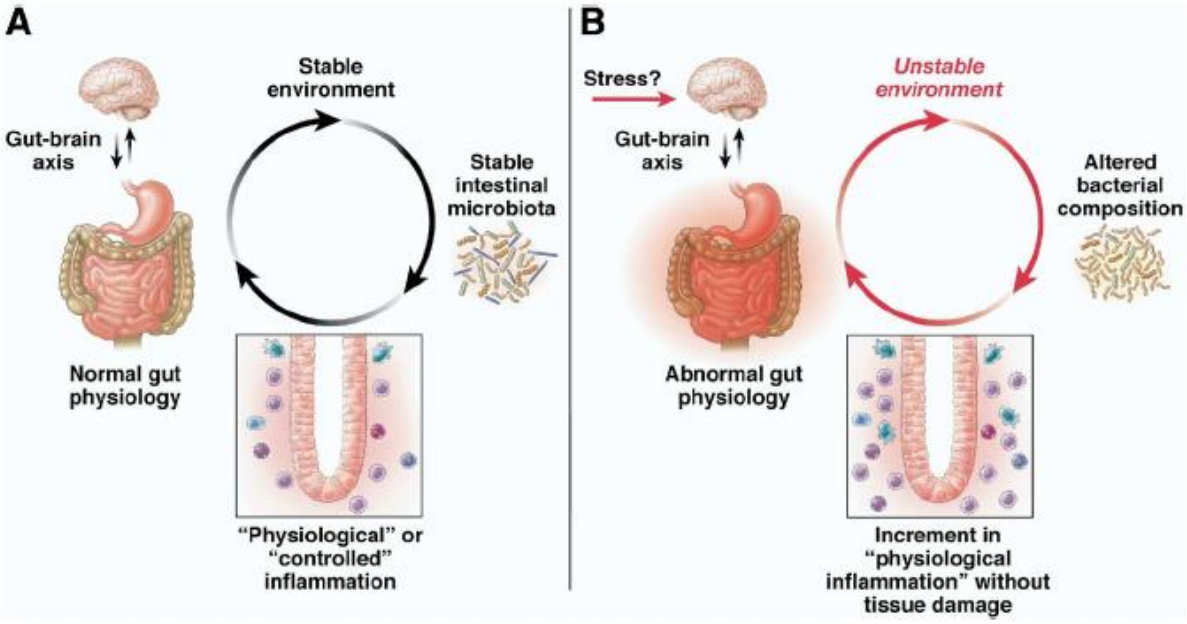

Figure 3: Stress and the gut microbiome (Collins et al., 2009; p.5)

Value of this research

Findings from this study may present important implications for ADHD management. Understanding the early interactions between ADHD in children, gut microbiome dysbiosis and environmental risk factors may open new avenues for preventative strategies and therapeutic interventions in at-risk populations (Cenit et al., 2018). Through refining the theoretical foundation of ADHD aetiology, specific risk factors can be identified which can inform more rigorous preventative strategies, whilst understanding the possible relationship between ADHD and gut dysbiosis in greater depth can open new avenues for more sustainable and integrative treatment strategies.

The gut microbiome is increasingly being considered as an important therapeutic target for a range of mental disorders (Allen et al., 2017; Bischoff, 2011; Cenit et al., 2017; Clapp et al., 2017; Dash et al., 2015; Dawson et al., 2016). Researchers are finding promising results in the treatment of mood disorders, anxiety disorders and certain neurodevelopmental disorders with specific strains of neuroactive, anti-inflammatory probiotics, which has been termed *psychobiotics* (Clapp et al., 2017; Dash et al., 2015; Zhou & Foster, 2015). Furthermore, in a longitudinal study by Partty et al. (2015) it was discovered that early probiotic supplementation reduced the risk of developing ADHD later in life, although more research was requested to understand the specific mechanisms involved.

The refinement of our understanding of the relationship between the gut microbiome and the brain present exciting new research possibilities, the findings of which could have important implications for ADHD conceptualisations in South Africa as well as on a global scale. South African researchers have found no significant socio-cultural differences in the presentation and prevalence of ADHD symptoms between various South African cultures and other Western cultures, which supports the common conceptualisation of a strong neurobiological foundation of ADHD as a global psychiatric disorder (Aase et al., 2006; Meyer et al., 2004).

We are thus presented with an opportunity to refine and enhance ADHD prevention and treatment strategies, not only within a South African context but also in the world at large. Ultimately, this study hopes to contribute towards a more comprehensive conceptualisation of the emerging relationship between ADHD and the gut microbiome through a specific focus on the relevant environmental factors and specific aetiological pathways involved.

Research Methodology

Research Problem and Objectives

Attention deficit hyperactivity disorder (ADHD) is currently the most prevalent neurodevelopmental disorder in the world with high comorbidity and a range of associated adverse outcomes. Preventative strategies and early identification with effective treatment interventions are therefore of vital importance and are to a large extent informed by aetiological conceptualisations.

Despite many years of research, however, ADHD still has a highly heterogenous clinical picture and the exact aetiological pathways remain unclear. In recent years there has been an exponential research interest on the association between neurodevelopmental disorders and the gut microbiome, with a clearly expressed need across multiple studies for researchers to investigate the emerging relationship between ADHD and gut microbiome dysbiosis to elucidate the specific mechanisms involved.

Research related to the gut-microbiome-brain connection has predominantly been conducted from a microbiological perspective and this interdisciplinary research study therefore aims to provide a humanistic approach with a clinical psychological lens to bridge theoretical gaps within the South African context.

The purpose of this study has therefore been to investigate the proposed link between ADHD and gut dysbiosis symptoms with a focus on the overlapping aetiological factors involved through the framework of the following objectives:

1. To investigate the relationships between previously identified environmental risk factors and ADHD symptom severity.

2. To investigate the relationship between symptoms of gut dysbiosis and ADHD symptom severity.

3. To investigate whether gut dysbiosis plays a mediating role in the effect of previously identified environmental risk factors on ADHD symptom severity.

Research Design

This study was conducted through the framework of a mixed methods research (MMR) approach with *pragmatism* as the overarching research paradigm (Morgan, 2007). MMR focuses on practical approaches using both quantitative methods (numbers) and qualitative methods (words) to solve a research problem, combining inductive and deductive logic through abductive thinking (Morgan, 2007). Mixed methods research can be defined as "research in which the investigator collects and analyses data, integrates the findings, and draws inferences using both qualitative and quantitative approaches or methods in a single study or a program of inquiry" (Creswell & Tashakkori, 2007, p. 4). According to Cresswell and Cresswell (2018; p.38), the core assumption of MMR is that "the integration of qualitative and quantitative data yields additional insight beyond the information provided by either the quantitative or qualitative data alone, allowing multiple ways to address a complex research problem as comprehensively as possible".

ADHD aetiology is a multifaceted and therefore complex field of inquiry, whilst gut microbiome research with children is a relatively recent research development with a lack of empirically tested assessment instruments. MMR was therefore deemed the most appropriate research design to address this complex research problem in a rigorous, comprehensive manner, offsetting weaknesses of quantitative and qualitative approaches used in isolation, providing stronger inferences, and enhancing the validity of the study through triangulation (Creswell & Creswell, 2018).

A non-experimental quantitative dominant convergent design (QUAN + qual) was applied with a cross-sectional, correlational focus to investigate the relationships between the dependent variable (ADHD symptom severity) and the independent variables (gut dysbiosis symptoms severity and environmental risk factors) (Creswell & Creswell, 2018; Stangor, 2015). Qualitative data was incorporated in the form of select open-ended questions to facilitate clarity and refinement of the quantitative research findings. MMR convergent design (figure 4) is a single-phase approach where quantitative and qualitative data are collected concurrently, analysed separately and then compared to determine whether the results confirm or disconfirm the research hypothesis (Creswell & Creswell, 2018).

Quantitative data serves to provide a general conceptualisation of the variables in question and the relationships involved between them, whilst qualitative data in the form of specific open-ended questions helps to refine and explain the statistical results (Morgan, 2007).

The study was conducted through the *ADHD Rating Scale IV*, the *Gastrointestinal Severity (GIS) Index* and a *self-compiled biographical questionnaire*, which took into consideration the various independent variables under investigation whilst incorporating open-ended questions to gather qualitative data pertaining to the quantitative findings.

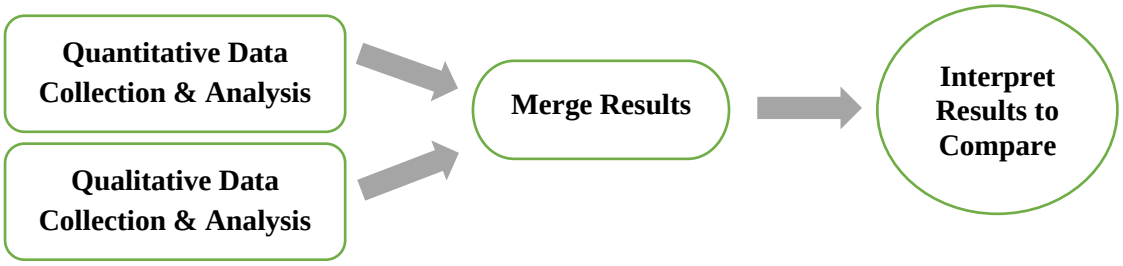

Figure 4: Convergent MMR Design (Creswell & Cresswell, 2018, p.284)

Participants and Sampling

South African children between the age of 6 and 12 who have been diagnosed with ADHD by a medical professional have formed the focus of this study, as this young population is still developing neurologically and therefore hold better prognostic potential than adolescents and adults. The children's parents have, however, answered the questions pertaining to their child as they possess the necessary knowledge and objectivity regarding developmental history and symptom presentation.

Participants have thus been included in the study on the bases of being the biological parent of a South African child between the age of 6 and 12 who has been diagnosed with ADHD by a qualified health professional (e.g., psychiatrist, psychologist, paediatrician). The researcher set out to reach approximately 200 participants and a total of 217 parents (one for each child) eventually filled in the online survey. Out of the 217 participants, 197 indicated that their child was diagnosed with ADHD by a medical professional, which automatically excluded 20 participants as a formal diagnosis was the essential inclusion criteria.

A non-probability convenience sampling strategy has been followed (Stangor, 2015). The Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA) and the ADHD South Africa Facebook group were utilised to attract research participants who were directed to Google Forms, a trusted survey site where they could complete the questionnaire in their own time from a location of their choice. This method has ensured minimal risk of COVID-19 exposure, ease of access, anonymity, as well as more efficient data gathering procedures and administration processes.

Table 1: Display of Demographic Variables According to Frequency

Demographic Variables			N	%
1	Gender	Male	136	67
		Female	61	31
2	Ethnicity	Caucasian	155	77.7
		African	20	10.2
		Coloured	18	9.1
		Other	4	2
3	Home Language	Afrikaans	103	52.3
		English	79	40.1
		Other African	15	7.6
4	Language of Education	English	102	51.8
		Afrikaans	93	47.2
		Other African	2	1

Sample population demographics for the children under investigation are illustrated in Table 1. The gender of the sample population consisted of 67% male (n=136) and 31% female (n=61), which is consistent with earlier findings of boys being at least twice as likely as girls to be diagnosed with ADHD (Reid et al., 2000; Rucklidge, 2010). Furthermore, the majority of the children in the sample were Afrikaans- (52.3%) and English-speaking (40.1%) Caucasian (77.7%) males between the age of 6 and 12. Demographically, this indicates that Caucasian males with English/Afrikaans as a first language are the most at risk of developing ADHD based on the research findings from this sample population.

Data Collection

The data was collected by means of three questionnaires, the *ADHD Rating Scale IV,* the *Gastrointestinal Severity (GIS) Scale* and a *self-report biographical questionnaire* (see Appendix A). These were administered in the form of an integrated online survey which was completed by participants on *Google Forms.* This allowed the participants to answer the questions in their own time at a location of their choice, ensuring ease of access, anonymity, and minimal risk of COVID-19 exposure. Once the data was collected in *Google Forms,* it was converted into *Microsoft Excel* format from which the qualitative data was transferred to *Microsoft Word* where it was coded and analysed thematically, whilst the quantitative data was transferred to *Statistical Package for the Social Sciences (SPSS) version 26* (IBM, 2019) for statistical analyses.

Measuring Instruments

The *ADHD Rating Scale IV* (Appendix A2) was used to measure ADHD symptom severity. It is an 18-item norm-referenced scale focussed on the ADHD diagnostic criteria for school-aged children and symptoms are assessed on a 4-point Likert scale with higher scores being indicative of more severe ADHD symptomology (Demaray et al., 2003). This instrument has displayed satisfactory internal consistency (Cronbach's alpha = 0.838) and test-retest reliability (0.773 - 0.887) in a trail conducted in 11 European countries, Australia, Israel, and South Africa (Zhang et al., 2005). In this study, both the Inattention subscale (Cronbach's alpha = 0.875) and the Hyperactivity-Impulsivity subscale (Cronbach's alpha = 0.873) showed good internal consistency reliability. The Total ADHD scale showed excellent internal consistency reliability with Cronbach's alpha = 0.917, where none of the individual items, if deleted, would have resulted in an increase in Cronbach's alpha (Table 2a).

Table 2 (a): Reliability Statistics for ADHD Rating Scale IV

Reliability Statistics

Cronbach's Alpha	N of Items
0,917	18

Item-Total Statistics

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
Fails to give close attention to details or makes careless mistakes in schoolwork.	33,88	100,285	0,446	0,916
Fidgets with hands or feet or squirms in seat.	33,76	100,153	0,472	0,915
Has difficulty sustaining attention in tasks or play activities.	33,90	98,068	0,597	0,913
Leaves seat in classroom or in other situations in which remaining seated is expected.	34,48	94,634	0,623	0,912
Does not seem to listen when spoken to directly.	34,15	95,613	0,639	0,911
Runs about or climbs excessively in situations in which it is inappropriate.	34,68	93,485	0,638	0,912
Does not follow through on instructions and fails to finish work.	34,05	96,490	0,644	0,911
Has difficulty playing or engaging in leisure activities quietly.	34,49	94,127	0,667	0,911
Has difficulty organizing tasks and activities.	34,11	97,776	0,609	0,912
Is always on the go or acts as if driven by a motor.	34,36	95,246	0,590	0,913
Avoids tasks (e.g., schoolwork, homework) that require sustained mental effort.	33,96	98,304	0,540	0,914
Talks excessively.	34,12	97,670	0,494	0,915
Loses things necessary for tasks or activities.	34,42	95,084	0,621	0,912
Blurts out answers before questions have been completed.	34,43	95,594	0,578	0,913
Is easily distracted.	33,71	99,471	0,603	0,913
Has difficulty awaiting turn.	34,14	94,297	0,648	0,911
Is forgetful in daily activities.	34,16	96,809	0,592	0,913
Interrupts or intrudes on others.	34,01	95,057	0,655	0,911

The *Gastrointestinal Severity (GIS) Index* (Appendix A3) was used as it was found to be the only relevant and feasible questionnaire available currently to measure gut dysbiosis symptoms severity with children, which may be due to the recency of this developing field of research. It was deemed appropriate as it consists of six of the most common symptoms

associated with gut dysbiosis and has been used prior in research pertaining to neurodevelopmental disorders (Adams et al., 2011; Kang et al., 2013; Ming et al., 2018). It therefore serves as a research proxy to assess for gut dysbiosis symptoms type and severity, determined on a 3-point Likert rating scale ranging from 0 ("none/normal") to 2 ("severe/significant"), where higher scores are indicative of more severe gut dysbiosis (Ming et al., 2018). In this study, the *GIS Index* showed poor internal consistency reliability, with Cronbach's alpha = 0.411 (Table 2b). However, given that the scale's items are not necessarily related to one another (e.g., having constipation does not increase your chance of having diarrhoea as well), internal consistency reliability may not be applicable to this scale. The question of this scale's validity also comes into question when working with a young population, whose gut dysbiosis symptoms may present in a much more subtle and indirect manner in comparison to the adult population and may therefore not be a clear representation of existing gut dysbiosis (Bischoff, 2011; Ming et al., 2018).

Table 2 (b): Reliability Statistics for Gastrointestinal Severity (GIS) Index

Item-Total Statistics and Cronbach's Alpha				
	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
Constipation (i.e., how frequently does your child "go to the toilet"?)	1,6630	2,658	0,042	0,473
Diarrhoea (i.e., how frequently does your child have a "runny tummy"?)	2,1215	2,974	0,192	0,390
Average Stool Consistency	2,0331	2,832	0,182	0,382
Stool Smell	1,8122	2,098	0,315	0,278
Flatulence (i.e., how often does your child struggle with gas?)	1,6519	1,973	0,306	0,280
Abdominal Pain (i.e., how often does your child complain of tummy aches?)	1,6851	2,428	0,216	0,354

A *self-report biographical questionnaire* (Appendix A1) was included to facilitate a more rigorous understanding of factors pertaining to gut dysbiosis to compensate for the perceived limitations of the *GIS Index.* It furthermore served to gather relevant biographical information with a focus on prominent environmental risk factors, which are prenatal maternal health (substance use, stress, obesity), prematurity, mode of delivery, infant health, early life nutrition and stress, and exposure to broad-spectrum antibiotics

and environmental toxins. The questionnaire consisted of closed- and open-ended questions, which aimed to provide explanatory qualitative data, which was triangulated by the researcher, the supervisor and co-supervisor to enhance qualitative validity, ensuring the information is credible, transferable, dependable, and confirmable (Creswell & Creswell, 2018).

Data Analysis and Interpretation

Convergent design data analysis occurred in three phases as outlined by Creswell and Creswell (2018). First, the quantitative database was statistically analysed using *Statistical Package for the Social Sciences (SPSS) version 26* (IBM, 2019). Descriptive statistics were calculated for the demographic variables, scales, and subscales. Cronbach's alpha coefficient has been calculated to determine the internal consistency of the scales used and Pearson's product-moment correlation coefficient was computed to investigate the direction and strength of the relationships between dependent and independent variables (Stangor, 2015). Hierarchical multiple regression analyses have been run to determine whether relationships exist between previously identified environmental risk factors and ADHD symptom severity, as well as between symptoms of gut dysbiosis and ADHD symptom severity, whilst controlling for confounding variables. The possible mediating role of gut dysbiosis in the relationship between previously identified environmental risk factors and ADHD symptom severity has been assessed by running a multiple regression with mediation analysis (Stangor, 2015).

Second, the qualitative responses were analysed by coding the data and collapsing the codes into themes. The MMR technique of *data transformation* was applied with six questions which necessitated the transformation of themes into quantitative data by grouping responses and counting them to obtain percentages (Creswell & Creswell, 2018). For example, participants were asked to name the medication and to describe observed side-effects, which were grouped and converted into percentages.

Third, the mixed methods data analysis consisted of integrating the two databases by merging the results from both the qualitative and the quantitative data and reporting the findings in a *side-by-side comparison* (Creswell & Creswell, 2018). The quantitative statistical results are reported first, followed by a discussion of the qualitative themes that either confirm or disconfirm the statistical results. A good example of this method of data interpretation can be seen in a study by McEvoy et al. (2018).

Ethical Procedure and Considerations

Ethical clearance was obtained from the General/Human Research Ethics Committee of the Faculty of Humanities, University of the Free State (Ethical clearance number: UFS-HSD2020/1384/0411) (Appendix E). The ethical guidelines set by the Health Professions Council of South Africa were adhered to, according to the Health Professions Act of 1974 (Government Gazette, 2006).

Once the study was able to commence, participants were located and given a clear, written explanation of what the study involves (Appendix B). They were assured that participation is completely voluntary and that they could withdraw at any time without any repercussions. They were also informed that in the unforeseen possibility of any emotional distress encumbered as a result of participating in the study, participants could be referred to the psychotherapeutic practice of the UFS master's program for debriefing or counselling.

The information gathered from participants has been kept strictly confidential and stored safely on a password protected laptop by the researcher, who has herself been diagnosed with ADHD as a child and therefore understands the ethical sensitivity and responsibility required in working with such a young and vulnerable population.

Parents were asked to answer the questions pertaining to their child as they possess the necessary knowledge and objectivity regarding developmental history and symptom presentation, whilst protecting the child from any possible embarrassment related to the questions posed about digestive symptoms. They have been asked to explain the purpose of the study in simple terms to their child, presenting them with the opportunity to decline their parent's participation on their behalf.

Given the challenging circumstances related to the recent COVID-19 pandemic, the study was conducted remotely on a secure online survey platform. We are aware that many South African parents may not have access to a printer and scanner, we therefore wanted to give participants the option to give consent digitally so as not to exclude any participants based on socioeconomic constraints.

Results

The mixed methods research (MMR) results from both quantitative and qualitative data analyses will be discussed by means of a side-to-side comparison in the following section to explore convergence of findings from both forms of inquiry and facilitate a comprehensive understanding of the variables under investigation (Creswell & Creswell, 2018). Firstly, however, the quantitative results from the descriptive statistics for the measuring instruments will be provided, followed by a correlational analysis, a hierarchical regression analysis and a mediation analysis, which will each be briefly discussed in turn.

Descriptive Statistics

The descriptive statistics were calculated using *Statistical Package for the Social Sciences (SPSS) version 26* (IBM, 2019) for the scales and subscales of the measuring instruments utilised in this study. The ADHD Rating Scale IV was statistically analysed according to the results from the two subscales (Inattention and Hyperactivity/Impulsivity) and from the total score for the combination of the two (see Tables 3a, 3b & 3c). Mean scores were obtained for the Inattention subscale (19.16), Hyperactivity/Impulsivity (17), and ADHD total score (36.13). The standard deviation for the Inattentive subscale was 5.23, for the Hyperactivity/Impulsivity was 6.11, and for the ADHD total score it was 10,38637 respectively. In terms of the descriptive statistics for the Gastrointestinal Severity (GIS) Index, a mean of 2.19 was obtained and a standard deviation of 1.81 (Table 4). To determine the skewness values, a range between -1 and +1 indicated slight skewness, values between -2 and +2 indicated moderate skewness (Peat et al., 2008). Normal distribution for kurtosis range between -3 and +3 (Brown, 1997). From the tables below, it is apparent that these values obtained for the scales and subscales all fall within the acceptable range prescribed for normality and that the data obtained can thus be used for further analyses.

Table 3 (a): Descriptive Statistics for Inattention Subscale: Means, Standard Deviations, Skewness and Kurtosis Values for the Total Participant Group (N=197)

			Statistic	Std. Error
Inattention subscale total score	Mean		19,1269	0,37313
	95% Confidence Interval for Mean	Lower Bound	18,3910	
		Upper Bound	19,8628	
	5% Trimmed Mean		19,2868	
	Median		20,0000	
	Variance		27,428	
	Std. Deviation		5,23715	
	Minimum		5,00	
	Maximum		27,00	
	Range		22,00	
	Interquartile Range		8,00	
	Skewness		-0,363	0,173
	Kurtosis		-0,601	0,345

Table 3 (b): Descriptive Statistics for Hyperactivity/Impulsivity Subscale: Means, Standard Deviations, Skewness and Kurtosis Values for the Total Participant Group (N=197)

			Statistic	Std. Error
Hyperactivity-Impulsivity subscale total score	Mean		17,0000	0,43523
	95% Confidence Interval for Mean	Lower Bound	16,1417	
		Upper Bound	17,8583	
	5% Trimmed Mean		17,1562	
	Median		17,0000	
	Variance		37,316	
	Std. Deviation		6,10871	
	Minimum		3,00	
	Maximum		27,00	
	Range		24,00	
	Interquartile Range		11,00	
	Skewness		-0,265	0,173
	Kurtosis		-0,784	0,345

Table 3 (c): Descriptive Statistics for ADHD total scale: Means, Standard Deviations, Skewness and Kurtosis Values for the Total Participant Group (N=197)

			Statistic	Std. Error
ADHD Total scale total score	Mean		36,1269	0,74000
	95% Confidence Interval for Mean	Lower Bound	34,6675	
		Upper Bound	37,5863	
	5% Trimmed Mean		36,3601	
	Median		37,0000	
	Variance		107,877	
	Std. Deviation		10,38637	
	Minimum		8,00	
	Maximum		54,00	
	Range		46,00	
	Interquartile Range		16,00	
	Skewness		-0,254	0,173
	Kurtosis		-0,649	0,345

Table 4: Descriptive Statistics for Gastrointestinal Severity (GIS) Index: Means, Standard Deviations, Skewness and Kurtosis Values for the Total Group (N=197)

			Statistic	Std. Error
GI Severity Index total score	Mean		2,1827	0,12889
	95% Confidence Interval for Mean	Lower Bound	1,9286	
		Upper Bound	2,4369	
	5% Trimmed Mean		2,0525	
	Median		2,0000	
	Variance		3,273	
	Std. Deviation		1,80902	
	Minimum		0,00	
	Maximum		8,00	
	Range		8,00	
	Interquartile Range		2,00	
	Skewness		0,805	0,173
	Kurtosis		0,242	0,345

Correlations between Criterion and Predictor Scores

The relationship between the predictor variables and the criterion variables were determined by running a Pearson's product-moment correlation, which was used to determine the strength and direction of these linear relationships. In this regard the following parameters were used: 0.1 = small effect, 0.3 = medium effect, and 0.5 = large effect (Peat et al., 2008). The correlational results are displayed in the tables below. From Table 5 (b) it is evident that there was a statistically significant positive association ($p < 0.05$) between Inattention subscale severity scores and GI Severity Index scores as assessed by Pearson's product-moment correlation coefficient ($r = 0.159$, $p = 0.026$). An increase in GI Severity index scores was thus associated with an increase in Inattention severity scores. There were no significant associat-ions between total ADHD severity scores and GI Severity Index scores ($r = 0.129$, $p = 0.070$) (Table 5c) or between Hyperactivity/Impulsivity severity scores and total GI Severity Index

scores (r = 0.084, p = 0.243) (Table 5a), as assessed by Pearson's product moment correlation coefficients.

Table 5 (a): Correlation between the Hyperactivity-Impulsivity subscale total score and the GI Severity Index total score

		Hyperactivity-Impulsivity subscale total score	GI Severity Index total score
Hyperactivity-Impulsivity subscale total score	Pearson Correlation	1	0,084
	Sig. (2-tailed)		0,243
	N	197	197
GI Severity Index total score	Pearson Correlation	0,084	1
	Sig. (2-tailed)	0,243	
	N	197	197

Table 5 (b): Correlation between the Inattention subscale total score and the GI Severity Index total score

		Inattention subscale total score	GI Severity Index total score
Inattention subscale total score	Pearson Correlation	1	.159*
	Sig. (2-tailed)		0,026
	N	197	197
GI Severity Index total score	Pearson Correlation	.159*	1
	Sig. (2-tailed)	**0,026**	
	N	197	197

*. Correlation is significant at the 0.05 level (2-tailed).

Table 5 (c): Correlation between the ADHD Total scale score and the GI Severity Index total score

		ADHD Total scale total score	GI Severity Index total score
ADHD Total scale total score	Pearson Correlation	1	0,129
	Sig. (2-tailed)		0,070
	N	197	197
GI Severity Index total score	Pearson Correlation	0,129	1
	Sig. (2-tailed)	0,070	

	N	197	197

Multiple Hierarchical Regression Analysis

Since the ADHD Inattention subscale showed a statistically significant correlation with gastrointestinal symptom severity, but not the ADHD Total scale or the ADHD Hyperactivity-Impulsivity subscale, it was decided to only probe the association between inattention and gut dysbiosis further.

A hierarchical multiple regression was run to determine whether independent variables related to gut dysbiosis (*Colic; Child antibiotics intake; Maternal antibiotic use during pregnancy; Mode of delivery; Breastfeeding; Formula milk intake; Intake of processed sugars/artificial colourants; Intake of gluten; Intake of dairy; Intake of Caffeine; Intake of MSG; Intake of raw fruit; Intake of raw vegetables; Water intake; Healthy general diet; Maternal obesity; GI Severity Index Total score)* made a statistically significant contribution to the prediction of inattention severity scores over and above the variance already explained by the following confounding variables: *Gender; Parents or siblings diagnosed with ADHD; Child diagnosed with another mental disorder; Exposure of child to toxins; Maternal tobacco use; Maternal alcohol use; Maternal stress during pregnancy; Premature birth; Labour complications; Separation of mother and child; Mother postnatal depression; Family conflict; Child exposed to stressful events.*

The most important measure for the interpretation of multiple hierarchical regression is R^2 ("R Square" column), which represents the percentage variation in the dependent variable explained by the independent variables. The significance of the difference that occurs in R^2 was then calculated using the hierarchical *F*-test and the effect sizes (f^2) were also determined. The aim was to determine whether the addition of independent variables related to gut dysbiosis (Model 2) resulted in a statistically significant increase in the prediction of ADHD Inattentive subscale scores when unrelated independent variables, which have previously been associated with the disorder, are controlled for (Model 1). From the cells highlighted in the Model Summary table below it can be seen that the addition of the variables related to gut dysbiosis in Model 2 did indeed statistically significantly improve the predictive power of the model, predicting 20% additional variance in ADHD Inattention symptom severity compared to Model 1 alone, R^2change = 0.200, F change (18, 158) = 2.428, p = 0.002 ($p < 0.01$).

Table 6: Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.280[a]	0,079	0,005	5,22319	0,079	1,073	14	176	0,385	
2	.528[b]	0,278	0,132	4,87896	0,200	2,428	18	158	0,002	2,159

From the highlighted block in Table 7, it can be seen that Model 2, which includes all confounding and all independent variables, did have a statistically significant effect on ADHD Inattention symptom severity, F (32, 158) = 1.904, p = 0.005.

Table 7: ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	409,676	14	29,263	1,073	.385[b]
	Residual	4801,585	176	27,282		
	Total	5211,261	190			
2	Regression	1450,183	32	45,318	1,904	.005[c]
	Residual	3761,078	158	23,804		
	Total	5211,261	190			

To determine which of the independent variables made statistically significant unique contributions to the prediction of scores on the Inattention subscale, the Coefficients table below was looked at. From the rows highlighted in Table 8, it is clear that the following four independent variables made statistically significant unique contributions to the prediction of Inattention symptoms severity, after all other variables were controlled for ($p < 0.05$): *the mother using tobacco during pregnancy, the mode of delivery, whether the child was breastfed, and the mother being mildly overweight during pregnancy.*

First, in comparison to mothers who never smoked during pregnancy, those who did report smoking scored 3.51 points higher on the ADHD Inattention symptom severity scale. Frequent maternal tobacco use during pregnancy was thus associated with increased ADHD Inattention symptom severity.

Second, compared to mothers who had a C-section, those who gave birth naturally scored 2.546 points higher on the ADHD Inattention symptom severity scale. Even though the majority of the child sample population were born via C-section, natural birth was found to be associated with an increase in ADHD Inattention symptom severity in the children.

Third, in comparison to children who were not breastfed, those who were breastfed scored 2.943 points lower on the ADHD Inattention symptom severity scale. Breastfeeding was thus associated with decreased ADHD Inattention symptom severity.

Finally, compared to mothers who were not overweight during pregnancy, those who were mildly overweight had children who scored 2.933 points higher on the ADHD Inattention symptom severity scale, while mothers who were overweight had children who scored 2.401 points higher on the ADHD Inattention symptom severity scale. Maternal prenatal obesity was thus associated with an increase in ADHD Inattention symptom severity in the children.

Table 8: Coefficients for Inattention Subscale

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95,0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
2	(Constant)	17,254	3,015		5,723	0,000	11,299	23,209					
	Gender	-1,100	0,825	-0,097	-1,332	0,185	-2,730	0,530	-0,077	-0,105	-0,090	0,856	1,168
	Has any of the parents or siblings been diagnosed with ADHD?	-0,385	0,809	-0,037	-0,476	0,634	-1,983	1,212	0,018	-0,038	-0,032	0,777	1,287
	Has your child ever been diagnosed with a mental disorder	1,017	0,787	0,095	1,291	0,199	-0,538	2,572	0,154	0,102	0,087	0,839	1,192
	Has your child ever been exposed to environmental toxins?	0,271	1,446	0,014	0,187	0,852	-2,585	3,127	-0,032	0,015	0,013	0,791	1,264
	Tobacco_use_Occasionally	1,489	1,310	0,084	1,136	0,258	-1,099	4,077	0,091	0,090	0,077	0,829	1,206
	Tobacco_use_Often	3,507	1,491	0,184	2,351	0,020	0,561	6,453	0,143	0,184	0,159	0,747	1,338
	Stress_pregnancy_Occasionally	-1,193	0,976	-0,165	-1,223	0,223	-3,121	0,734	-0,011	-0,097	-0,083	0,251	3,984
	Stress_pregnancy_Often	1,355	1,588	0,117	0,853	0,395	-1,782	4,492	0,047	0,068	0,058	0,244	4,098
	Was your child born prematurely?	0,585	1,005	0,044	0,582	0,561	-1,400	2,570	0,075	0,046	0,039	0,790	1,266
	Were there any labour	-0,529	0,898	-0,045	-0,589	0,557	-2,303	1,245	0,004	-0,047	-0,040	0,790	1,266
	During the first 3 years of your child's life, how frequently was the mother and infant separated?	-1,992	1,236	-0,131	-1,612	0,109	-4,433	0,449	-0,003	-0,127	-0,109	0,687	1,456
	During the first 3 years, how often did the mother struggle with post-natal depression?	-0,738	1,305	-0,045	-0,565	0,573	-3,316	1,841	0,055	-0,045	-0,038	0,709	1,410
	During the first 3 years, how frequently was he/she exposed to any other significantly stressful life events?	-1,240	1,352	-0,076	-0,917	0,361	-3,910	1,431	-0,009	-0,073	-0,062	0,661	1,512
	During the first 3 years, how frequently was he/she exposed to significant family conflict?	2,157	1,245	0,142	1,733	0,085	-0,302	4,615	0,059	0,137	0,117	0,677	1,477
	Did your child ever have colic?	-0,055	0,864	-0,005	-0,063	0,949	-1,762	1,652	0,050	-0,005	-0,004	0,783	1,277
	Did your child take any antibiotics during the first 3 years of life?	2,061	1,196	0,134	1,722	0,087	-0,302	4,423	0,189	0,136	0,116	0,757	1,321
	How frequently did the biological mother use antibiotics during pregnancy?	1,651	1,066	0,121	1,549	0,123	-0,454	3,756	0,079	0,122	0,105	0,745	1,342
	Mode of delivery: Reference category: C-section	2,546	0,890	0,228	2,860	0,005	0,788	4,305	0,111	0,222	0,193	0,719	1,391
	Was your child breastfed? Reference category: No	-2,943	0,882	-0,240	-3,338	0,001	-4,684	-1,202	-0,217	-0,257	-0,226	0,883	1,132
	Did your child drink formula milk?	-1,535	1,578	-0,073	-0,973	0,332	-4,651	1,581	-0,021	-0,077	-0,066	0,813	1,231
	Processed sugar & artificial	-0,523	0,877	-0,050	-0,597	0,551	-2,255	1,208	0,035	-0,047	-0,040	0,650	1,538
	Gluten	1,049	1,078	0,080	0,973	0,332	-1,080	3,178	0,183	0,077	0,066	0,673	1,486
	Dairy	1,683	1,074	0,127	1,567	0,119	-0,438	3,804	0,142	0,124	0,106	0,691	1,446
	Caffeine	-1,436	1,622	-0,066	-0,885	0,377	-4,638	1,767	-0,004	-0,070	-0,060	0,825	1,213
	MSG	-1,193	0,884	-0,109	-1,350	0,179	-2,938	0,553	0,035	-0,107	-0,091	0,703	1,423
	Raw fruit	-0,506	1,025	-0,044	-0,494	0,622	-2,530	1,518	-0,035	-0,039	-0,033	0,588	1,700
	Raw vegetables	0,626	0,917	0,059	0,683	0,496	-1,186	2,438	0,001	0,054	0,046	0,608	1,646
	Water	0,120	1,009	0,009	0,119	0,905	-1,873	2,113	-0,021	0,009	0,008	0,799	1,251
	How healthy do you perceive your child's general diet to be on a scale from 1 to 5?	-0,060	0,544	-0,010	-0,110	0,913	-1,133	1,014	-0,052	-0,009	-0,007	0,562	1,778
	Obesity_Mildly_overweight	2,933	0,927	0,252	3,165	0,002	1,103	4,763	0,125	0,244	0,214	0,719	1,390
	Obesity_Overweight	2,401	1,106	0,174	2,172	0,031	0,218	4,585	0,051	0,170	0,147	0,711	1,406
	GI Severity Index total score	0,415	0,218	0,144	1,905	0,059	-0,015	0,846	0,159	0,150	0,129	0,805	1,243

a. Dependent Variable: Inattention subscale total score

Mediation Analyses

Following from the hierarchical regression analysis, the study explored the possible mediating role of gut dysbiosis symptoms between ADHD Inattentive symptoms and the four statistically significant independent variables (i.e., environmental risk factors). Hence, it was investigated whether gut dysbiosis (as measured by the GIS Index) mediates the relationships found between *Inattention symptom severity* (DV) and *maternal tobacco use*, *mode of delivery*, *breastfeeding* and *maternal obesity* (IV). According to Stangor (2015), three conditions must be met before it can be determined whether mediation has occurred. First, the independent variable must predict the dependent variable, and from the hierarchical regression analysis it is evident that this first condition has been met for all four variables. Second, the independent variable must predict the mediator (GIS Index total score), and finally, the mediator must predict the dependent variable (Stangor, 2015). Each of these independent variables have been analysed for mediation and each will be discussed in turn.

First looking at maternal tobacco use, an independent samples T-test was conducted to determine if a significant relationship exists between *maternal tobacco use during pregnancy* and *GIS total score*. The assumption of equality of variance was not violated, $F = 2.170$, $p > 0.05$, and therefore results in the Equal variances assumed row were interpreted. It was found that there was not a significant difference in the mean GIS scores of mothers who did not smoke or only smoked occasionally during pregnancy (Mean = 2.13) and mothers who smoked often during pregnancy (Mean = 2.81), $t(194) = -1.433$, $p = 0.153$. The second condition for mediation is therefore not met. Since the mediator (GIS total) was not significantly associated with the IV (maternal smoking), it is not possible for GIS total score to mediate the relationship between maternal smoking during pregnancy and Inattention symptom severity.

Table 9 (a): Independent Samples Test for Maternal Tobacco Use

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
GI Severity Index total score	Equal variances assumed	2,170	0,142	-1,433	194	0,153	-0,67361	0,47010	-1,60077	0,25355
	Equal variances not assumed			-1,177	16,710	0,256	-0,67361	0,57219	-1,88242	0,53520

Second, an independent samples T-test was conducted to determine whether a significant relationship exists between *mode of delivery* and *GIS total score* (Table 9b). The assumption of equality of variance was not violated, F=0.632, p > 0.05, and therefore results in the Equal variances assumed row is interpreted. No significant difference was found in the mean GIS scores of mothers who gave birth naturally (Mean = 2.4127) and mothers who had a C-section done (Mean = 2.0530), t (193) = -1.323, p = 0.187. The second condition for mediation is therefore not met. Since the IV (mode of delivery) was not significantly associated with the mediator (GIS total), it is not possible for GIS total score to mediate the relationship between mode of delivery and Inattention symptom severity.

Table 9 (b): Independent Samples Test for Mode of Delivery

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the	
									Lower	Upper
GI Severity Index total score	Equal variances assumed	0,632	0,428	-1,323	193	0,187	-0,35967	0,27177	-0,89568	0,17635
	Equal variances not assumed			-1,289	114.42	0,200	-0,35967	0,27896	-0,91226	0,19293

Third, an independent samples T-test was conducted to determine if a significant relationship exists between *breastfeeding* and *GIS total score*. The assumption of equality of variance was not violated, F=0.172, p > 0.05, and therefore results in the Equal variances assumed row is interpreted. Here again no significant difference was detected in the mean GIS scores of mothers who breastfed their children (Mean = 2.2067) and mothers who did not (Mean = 2.1064), t (195) = -0.331, p = 0.741. The second condition for mediation is thus not met. Since the IV (breastfeeding) was not significantly associated with the mediator (GIS total), it is not possible for GIS total score to mediate the relationship between breastfeeding and Inattention symptom severity.

Table 9 (c): Independent Samples Test for Breastfeeding

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
GI Severity Index total score	Equal variances assumed	0,172	0,679	-0,331	195	0,741	-0,10028	0,30309	-0,69804	0,49747
	Equal variances not assumed			-0,313	70,992	0,755	-0,10028	0,32002	-0,73838	0,53781

Finally, a one-way analysis of variance (ANOVA) was conducted to determine if a significant relationship exists between *maternal obesity during pregnancy* and *GIS total score* (Table 10b). Here again no significant differences were found in the mean GIS scores between mothers who were *Not at all overweight* (Mean = 2.1308), *Mildly overweight* (Mean = 2.0909), and *Overweight* (Mean = 2.5588) (Table 10a), F (2, 193) = 0.847, p = 0.430 (Table 10b). The second condition for mediation is therefore not met. Since the IV (*maternal obesity during pregnancy*) was not significantly associated with the mediator (*GIS total*), it is not possible for GIS total score to mediate the relationship between maternal obesity during pregnancy and Inattention symptom severity.

Table 10 (a): Descriptive Statistics for Maternal Prenatal Obesity and GIS total score

		GI Severity Index total score						
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Not at all	107	2,1308	1,79141	0,17318	1,7875	2,4742	0,00	8,00
Mildly overweight	55	2,0909	1,72426	0,23250	1,6248	2,5570	0,00	7,00
Overweight	34	2,5588	1,98771	0,34089	1,8653	3,2524	0,00	7,00
Total	196	2,1939	1,80687	0,12906	1,9393	2,4484	0,00	8,00

Table 10 (b): ANOVA: Relationship between GIS total score and Maternal Obesity

GI Severity Index total score					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5,537	2	2,768	0,847	0,430
Within Groups	631,096	193	3,270		
Total	636,633	195			

Convergent Results Interpretation and Discussion

Mixed methods research (MMR) in the form of a convergent design is conducted within a single-phase where quantitative and qualitative data were collected concurrently in an online survey, after which the data was analysed and then compared to determine whether the results confirm or disconfirm the research hypothesis (Creswell & Creswell, 2018). The qualitative data obtained from open-ended questions was analysed thematically and *data transformation* was applied by grouping responses and counting them to obtain percentages (Appendix D). The results obtained will now be discussed in conjunction with the qualitative data in an integrative manner through a *side-by-side comparison* (Creswell & Creswell, 2018).

Diagnostic Information

Parent participants were asked whether their child was diagnosed with ADHD by a professional medical/mental health practitioner. From the 217 sample, only 197 participants answered "Yes", which automatically excluded 21 participants as this was an essential inclusion criterion. Of this remaining sample population, most reported formal diagnosis by a paediatrician (N = 66; 33.5%), clinical/counselling psychologist (N = 49; 24.9%), and a psychiatrist (N = 44; 22.3%). Next, participants were asked about the age of diagnosis and from the range of responses a mean age of 6.17 years were obtained (Table 11). According to the DSM-V criteria (APA, 2013), several symptoms need to be present before age 12 to convey substantial clinical presentation, although ADHD symptoms are often identified during school years when they become more prominent and impairing. It would thus be logical to conclude that most children in the sample were diagnosed after entering school around the age of six.

Table 11: Age of Diagnosis

N	Valid	196
	Missing	1
Mean		6,1735
Median		6,0000
Mode		6,00
Std. Deviation		1,51252
Range		9,00
Minimum		3,00
Maximum		12,00

Apart from a diagnosis of ADHD, 39.6% (N = 78) of the children included in the study were reported to have a comorbid diagnosis. The most prominent psychiatric comorbidities were anxiety-related disorders (N = 46; 60%), oppositional defiant disorder (N = 15; 19.2%), autism spectrum disorder (N = 13; 16.7%), sensory processing disorder (N = 11; 14.1%), depression (N = 9; 11.5%), and specific learning disorder (N = 5; 6.1%), which confirms previous research findings (Akmatov et al., 2019; Bakare, 2012; Caye et al., 2016; Connor et al., 2010; Kessler et al., 2014; Pliszka, 2000). Researchers hold that many children with ADHD have at least one comorbid psychiatric disorder, known as "complex" ADHD, which further complicates diagnostic and treatment strategies (Biederman et al., 1992; Connor et al., 2010; Seymour & Miller, 2017).

Medication and Side Effects

Most parents in the sample indicated that their child is currently on ADHD medication (N = 168; 85.3%). Methylphenidate in the form of Ritalin (N = 104; 61.9%), Concerta and its generic version, Neucon, coming in second (N = 56; 33.3%) is clearly the most frequently prescribed medication (Table 5a). Parent participants listed a wide range of side-effects from ADHD medication as reported by their child and/or observed by the parent themselves (Table 5b). Most complained of a significant decrease in appetite (N = 85; 50.6%) as a side-effect from ADHD medication, and when asked directly whether the child struggles with his/her appetite, only 26 (13.2%) answered "never" and 31 participants (15.7%) answered "always". This may be further complicated by most children in the sample being described as picky eaters (N = 151; 90.3%).

Several parents reported that their children struggle with stomach cramps as a side-effect of the ADHD medication (N = 14; 8.3%), for example: *"When taking meds he gets stomach cramps and discomfort"*. It is relevant to note that stomach cramps or abdominal pains can also be a symptom of gut dysbiosis (McKeown et al., 2013) and may also be attributable to anxiety (N = 10): "*Anxiety due to ADHD causes tummy ache"; "When worked up or bad day will complain of sore stomach"*.

	Table 12 (a): ADHD Medication	**N**	**%**
1	Ritalin	104	61.9
2	Concerta	28	16.6
3	Neucon	28	16.6
4	Risperdal	23	13.7
5	Contramyl	9	5.4
6	INIR	9	5.4
7	Strattera	6	3.6
	Total Valid (N)	168	100
	Total Missing (N)	29	

	Table 12 (b): ADHD Medication Side Effects	**N**	**%**
1	Decreased appetite	85	50.6
2	Insomnia	27	16.1
3	Emotional/mood swings	15	8.9
4	Stomach cramps	14	8.3
5	Anger/aggression/irritation/frustration	14	8.3
6	Anxiety	10	6
7	Apathy (withdrawn/spacey/zombie-like)	9	5.4
8	Weight gain	8	4.8
9	Depression	7	4.2
10	Drowsiness/loss of energy/lethargy	6	3.6
	Total Valid (N)	168	100
	Total Missing (N)	29	

Mode of Delivery, Gestation Age and Birthweight

A relatively small portion of parents indicated that their child was born prematurely (N = 38; 19.3%) and no significant associations were found between gestation age and ADHD symptom severity. It is, however, important to note that premature birth has been associated with ADHD aetiological risk and this may have played a role in the development of ADHD symptoms in this portion of children (Franz et al., 2017). Furthermore, the mean of the

indicated birthweights of the infants were 3.22kg (Table 13), which falls in the normal weight range (Franz et al., 2017).

Table 13: Statistical analysis of birthweights		
N	Valid	189
	Missing	8
Mean		3,2260
Median		3,2000
Mode		3,60
Std. Deviation		0,69098
Range		6,94
Minimum		1,06
Maximum		8,00

In terms of mode of delivery, the majority of the research population indicated that their children were born via a C-section (N = 132; 67%), substantiating the hypothesis of C-section births being a risk factor for the development of ADHD, which may be due to the impact on gut microbiome development as shown in previous research findings (Hoang et al., 2020; Nagpal & Yamashiro, 2017). In comparison to mothers who had a C-section birth, however, those who gave birth naturally scored 5.09 points higher on the Inattention symptom severity subscale. Giving birth naturally was thus associated with an increase in Inattention symptom severity in the children, which contradicts earlier findings (Hoang et al., 2020; Nagpal & Yamashiro, 2017; Tribe et al., 2018).

It is worthy to note that 23.8% of mothers who had a natural birth reported birth complications and 11.9% gave birth prematurely, which have been associated with ADHD aetiology (Cortese & Coghill, 2018; Franz, 2017; Lim et al., 2018; Wagner et al., 2009). Secondly, 22.3% of these mothers reported struggling with constipation, whilst 20.8% reported struggling with urinary tract infections, proven symptoms of microbiome dysbiosis (Finucane, 2017; Ohkusa et al., 2019). Lastly, 10.4% reported taking antibiotic medication during pregnancy, which enhances the risk of dysbiosis (Dinsmoor et al., 2005). One possible conclusion which may therefore be drawn is that of the mothers (32%) who gave birth naturally, some could have suffered from dysbiosis and may thus have transferred this dysbiotic microbiome profile to the infants during birth.

Maternal Substance Use, Environmental Toxins and Antibiotics

In comparison to mothers who never smoked during pregnancy (N = 161; 81.7%), those who indicated that they did smoke often (N = 16; 8.1%) scored 6.29 points higher on the Inattention symptom severity scale. Frequent tobacco use during pregnancy was thus associated with increased Inattention symptom severity, confirming earlier research findings of maternal smoking as a significant risk factor in ADHD aetiology (Cortese & Coghill, 2018; Eilertsen et al., 2017; Nigg, 2012; Sciberras, 2017; Sharma, 2013; Tarver, 2014; Thakur et al., 2012; Thapar, 2012).

A small portion of participants indicated occasional maternal prenatal alcohol use (N=18; 9.1%) and an even smaller portion indicated that alcohol was often consumed during pregnancy (N = 4; 2%). In terms of environmental toxins, 8.1% (N = 16) reported early exposure of the infant to pesticides (N = 8); vaccines (N = 3); general toxins in products (N = 2); and methamphetamine (N = 1). It is worthy to note that 17.8% (N = 35) of the participants indicated at least one course of maternal antibiotic treatment during pregnancy (Table 14). Even though no significant association with ADHD symptom severity was found, the impact on maternal microbiome health should still be considered in relation to the infants' gut microbiome development.

Table 14: *Frequency of Maternal Prenatal Antibiotic Exposure*		N	%
Valid	Never	160	81,2
	Occasionally	33	16,8
	Often	2	1,0
	Total	195	99,0
Missing	System	2	1,0
Total		197	100,0

Maternal Health and Obesity

In comparison to biological mothers who indicated that they were not overweight during pregnancy (N = 107; 54.3%), those who were mildly overweight (N = 55; 27.9%) had children who scored 4.50 points higher on the Inattention symptom severity subscale. Mothers who reported being mildly overweight during pregnancy was thus associated with an increase in Inattention symptom severity in the children. This finding confirms previous research associations found between high body mass index (BMI) / obesity and increased risk of ADHD

development (Bazar et al., 2006; Cerdo et al., 2016; Li et al., 2020; Rivera et al., 2015; Rodriguez et al., 2007).

In addition, 40% (N = 79) of the biological mothers struggled with health problems during pregnancy. Interestingly, most of their reported conditions have been associated with microbiome dysbiosis, such as constipation (N = 28) (Ohkusa et al., 2019); urinary tract infections (N = 24) (Finucane, 2017); diabetes (N = 4) (Cortez et al., 2019; Vallianou et al., 2018); hypertension (N = 4) (Li et al., 2017; Richards et al., 2017); anaemia (N = 2) (Stelle et al., 2019), high blood pressure (N = 9); diarrhoea (N = 1) (Bischoff, 2011); thrush/vaginal/yeast infection (N = 4) (Bertolini et al., 2019); and depression (N = 4) (Capuco et al., 2020). These aforementioned maternal health problems may therefore indicate possible maternal microbiome dysbiosis which could have been transferred to the infant during the perinatal period.

Immunity and Inflammation

Parent participants were asked to list any co-occurring physical health problems in their child, which included several conditions associated with gut dysbiosis, such as eczema (N = 17) (Marrs & Flohr, 2016); allergies (N = 26) (Prince at al., 2015); asthma (N = 14) (Sbihi et al., 2019); sinusitis (N = 35) (Lee et al., 2016); allergic rhinitis (N = 3) (Ni et al., 2019); food intolerances (N = 3) (Hill et al., 2016); and chronic constipation (N = 5) (Mancabelli et al., 2017). In addition, many children in the sample were reported to have suffered from colic as an infant (N = 60; 30.5%). According to Rhoads et al. (2018), colic is linked to gut inflammation and dysbiosis, which further substantiates the research hypothesis.

The range of health problems reported may explain why the majority of children in the study (N = 170; 86.3%) were given regular courses of antibiotic treatments during their early stages of development. For example, one mother reported: "*Between ages 0-3 at least 4 doses per year",* whilst another said: "*Up to 3 years of age, he had a lot of antibiotics almost every second month*". Compared to children who did take antibiotics during the first three years of life, those who did not take antibiotics scored 5.15 points lower on the Inattention symptom subscale. Taking antibiotics during the first three years of life was thus associated with increased Inattention symptom severity, which confirms previous research findings (Aversa et al., 2020; Slykerman et al., 2019).

This situation is evidently exacerbated by the continuation of regular courses of antibiotic treatments. Parents were asked: "On average, how many courses of antibiotics does your child take per year?", which revealed that only 12 children (6%) are not given antibiotics on a regular basis. Most children are currently still receiving one to seven courses of antibiotics every year. For example, one mother reported: *"Mostly he was feverish so was always given antibiotics."*

It has been well established that the administration of antibiotic treatments can drastically disrupt the intricate microbial balance in the intestinal lining, frequently resulting in gut dysbiosis (Sharon et al., 2016). Antibiotics are, however, generally the first line of treatment for many ailments (Neuman et al., 2018), and the children in this study evidently struggled with a range of health problems which may explain why they frequently received antibiotic treatments: *"Hardly any (courses of antibiotics) now but as a baby he was sick all the time."* "*Many (courses of antibiotics) due to the eczema".* This statement by a parent about antibiotic treatments furthermore indicates the suspected association between antibiotics, gut dysbiosis and related symptoms such as constipation: *"Now, about zero. Between the ages of 1.5 and 3 - around 2 courses every 5 months due to reoccurring tonsillitis. We assume that this is the reason for her constipation."*

Early Life Nutrition and Childhood Diet

The majority of children (N = 150; 76.1%) were reported to have been breastfed for a period ranging from 1 week to 2 years. In comparison to children who were not breastfed, those who were breastfed scored 4.93 points lower on the Inattention symptom severity subscale. Breastfeeding was thus associated with decreased Inattention symptom severity, which confirms previous research findings (Mimouni-Bloch et al., 2013; Stadler et al., 2016; Tseng et al., 2018). Breastmilk is rich in probiotics and a key contributor to the development of a healthy gut microbiome (Cerdo et al., 2016; Lemas et al., 2016; Pannaraj et al., 2017), which may explain why breastfeeding is an important protective factor in ADHD aetiology.

The gut microbiome is furthermore significantly influenced by a person's daily diet (Dam et al., 2019). Hence, this study also included related questions, which revealed that many of the children in this sample regularly consume known gut irritants and microbiome *eubiosis* disrupters (Wang et al., 2020), the most prominent of which are processed sugar and artificial colourants (N = 171; 86.5%;), gluten (N = 187; 95%), and dairy (N = 183; 92.9%). From the

qualitative responses, it is evident that dietary factors have a noticeable impact on mood, behaviour, cognitive functioning, and ADHD symptoms severity:

- *"I realized this a year ago. Unhealthy diet... high carbs and high sugar intake made him less focused and more irritable. We changed our whole family's diet, and he has also been off all medication for 10 months now, just by changing his diet."*
- *"He seems more hyper and struggles to focus when he eats gluten and sugar."*
- *"Concentration and any form of schoolwork and emotional instability is worse when she has eaten sugar, milk, wheat or gluten."*
- *"Dairy, colourants additives and preservatives make things worse."*
- *"Hyper from fizzy drinks."*
- *"I have decreased gluten and sugar hugely and have found that she complains less about thrush (i.e., a symptom of yeast/Candida infection)."*
- *"Sugar causes major irritability, frustration and affects concentration."*
- *"We have changed our diet by cutting out gluten and dairy on top of sugar. He seems less impulsive and more cooperative. There has also been no incidents at school the last two weeks. Whereas previously, there were at least two a week."*

Urban Western diets are generally high in processed sugars and saturated fats, and major contributions of gut dysbiosis (Cenit et al., 2017; Noble et al., 2017). Conversely, rural and traditional diets, generally characterized by unprocessed and fermented foods, are associated with more diverse and balanced gut microbiomes (i.e., *eubiosis*) (Cenit et al., 2017; Noble et al., 2017). In this sample population, some parent participants indicated that their child eats raw fruit (N=76; 38.6%) and raw vegetables (N=33; 16.8%) on a daily basis. Research has shown that natural fibres in fruit and vegetables are essential for a healthy gut microbiome (Dam et al., 2019). Although, encouraging healthy dietary patterns may be complicated when the majority of children in this sample have been described as "picky eaters" (N=151; 76.7%).

Looking at early life nutrition in terms of supplements, the study asked parent participants to list any supplements their child takes on a regular basis. Parents listed a wide range of supplements, the most relevant of which will be discussed briefly. First, 46 participants (23.4%) reported that their child is taking omega-3 supplements on a regular basis, which has proven efficacy in the treatment for ADHD (Richardson, 2006). This may be due, in part, to the benevolent impact on the gut microbiome as discussed in the literature review (Constantini et al.; 2017; Noriega et al., 2016). Secondly, 15 participants (7.6%) noted regular

microflora/probiotic supplementation. According to a systematic review by Kalenik et al. (2021), research on the treatment efficacy of probiotic supplements in ADHD remains inconclusive. The qualitative data, however, revealed some interesting observations:

- *I hugely believe in the gut being linked to mental health. I need to have her on a probiotic more consistently to give a better evaluation.*
- *Probiotics do him good.*
- *He seems to be a little bit more calm when consistently taking probiotics.*
- *It is difficult to quantify as I know his diet is not conducive to good gut health. That is why I insist on the microflora supplement.*
- *Before medicine for ADHD, he was constipated and picky eater. When he was 5, I gave him probiotics regularly and I feel that definitely improved the bowels. I would have carried on, but expenses are a factor.*

The last statement presents the important consideration of the cost of probiotic supplements, which appears not to be acknowledged in international studies where this often presented as a possible solution to ameliorating gut dysbiosis (Stevens et al., 2019). This option is not as feasible in a country such as South Africa, where a large portion of the population lives with a low socio-economic status and increasingly struggle with food insecurity on a daily basis (Hendriks, 2014). It is logical to assume that many South African parents may thus not be able to afford daily probiotic supplements or a healthy diet rich in fresh produce.

The option of incorporating fermented foods, for example *sauerkraut* (i.e., cultured cabbage) and *amasi* (i.e., cultured milk), can thus be considered as more cost-effective, sustainable alternatives (Franz, 2014; Khumalo, 2007; Selhub, Logan & Bested, 2014). Recent research has shown that the use of fermented foods in diets did confer gastrointestinal and cognitive benefits due to the beneficial impact on the health of the gut microbiome. (Clapp et al., 2017). Families may therefore be presented with the option to make more use of natural sources of probiotics in the form of fermented foods, which may be a more sustainable, cost effective prevention and treatment option for gut microbiome dysbiosis.

Prenatal Maternal Stress and Early Life Stressors

The study found that 47.2% (N=93) of the biological mothers occasionally experienced abnormally intense stress during pregnancy and 27.9% (N=55) often experienced abnormally intense prenatal stress. The majority of biological mothers (75%) therefore experienced

abnormal stress during pregnancy, which corresponds with other research studies finding a correlation between ADHD and maternal prenatal stress (Grizenko et al., 2015; Okano et al., 2018; Rodriguez & Bohlin, 2005; Ronald et al., 2011; Sciberras, 2017).

In terms of postnatal early life stressors, we will look at the most commonly identified risk factors associated with ADHD. First, 45 (22.8%) participants reported occasional mother-infant separation, whilst 27 (13%) indicated that the mother and infant were often separated. According to Manyema & Richter (2019), mother-infant separation has been associated with ADHD and is understood as a significant early life stressor with a range of neurodevelopmental and psychosocial implications. Secondly, 61 (31%) participants reported occasional post-natal depression and 23 (11.7%) reported that the mother often struggled with postnatal depression, which has previously been associated with ADHD (Humphreys et al., 2018). Moreover, in terms of early exposure to significant family conflict, 65 (33%) participants indicated occasional exposure, whilst 27 (13.7%) reported that the infant was often exposed to this familial stressor. Finally, participants were asked to rate the frequency of early exposure to significantly stressful life events, such as divorce, death of a loved one, and home relocation, to which 84 (42.6%) indicated occasional exposure and 23 (11.7%) reported that the infant was often exposed.

Furthermore, the frequency of early hospitalisation was investigated, and 90 (45.7%) participants indicated that the child was occasionally hospitalised whilst 16 (8.1%) participants indicated that the child was often hospitalised. Early hospitalisation can also be a significant stressor for a young child: *"He had intussusception and appendix surgery when he was 2.5 years",* while one parent reported that the child "*Had issues with underdeveloped nerves in his intestine and had to have surgery at a year old for it."*

Looking at all these postnatal early life stressors combined, a total of 116 (58.9%) children in the study was often exposed to one or a combination of these psychosocial risk factors, all of which have been found to enhance the risk of developing ADHD (Humphreys et al., 2018; Nigg et al., 2020). It has been established that severe, chronic stress influences neurodevelopment and the gut microbiome (Cong et al., 2015; Foster et al., 2017; Rakers et al., 2017; Rosin et al., 2020; van den Bergh et al., 2017; Vogel et al., 2020), however, the psychosocial impact of this is far reaching with a range of developmental implications which will need to be investigated in further research studies.

Gut Dysbiosis Symptoms

The *6-item Gastrointestinal Severity (GIS) Index* was used as a research proxy as it was the only existing measure of gut microbiome dysbiosis symptoms which was deemed feasible and relevant for the focus of this study. Measuring gut dysbiosis symptoms with a questionnaire appears to be a new development, which may be attributed to the recency of this emerging field of gut-microbiome-brain research with the paediatric population. Due to the questionable reliability and validity of the *GIS Index* as a measuring instrument, it was decided to include relevant open- and closed-ended questions related to gut health to compile a more comprehensive clinical picture.

Participants were asked if any other digestive symptoms are present apart from those measured in the *GIS Index*, to which 39 (19.8%) answered "yes" and listed the symptoms in the table below. They were also asked to list any formally diagnosed gastrointestinal disorders to which one parent gave the following response: "*Not yet but I want him to see a gastro-enterologist".* These questions revealed that at least 37 (18.8%) of the children in this sample experience other gastrointestinal symptoms than those listed in the *GIS Index*, and 23 (11.7%) have received formal gastrointestinal diagnoses by a medical professional. It is thus evident that the measuring instrument utilised in this study insufficiently accounts for the range of symptoms necessary to obtain more conclusive results.

	Table 15 (a): Other Gastrointestinal Symptoms	**N**	**%**
	Nausea	18	46.2
	Vomiting	8	20.5
	Faecal incontinence	4	10.3
	Indigestion	3	7.7
	Excessive burping	1	2.6
	Frequent defaecation	1	2.6
	Heartburn	1	2.6
	Acidity	1	2.6
	Total	37	100

	Table15 (b): Gastrointestinal Disorders	**N**	**%**
	Irritable Bowel Syndrome (IBS)	5	21.7
	Encopresis	4	17.4
	Spastic colon	3	13
	Chronic constipation	2	8.6
	Reflux	3	13
	Gut inflammation	1	4.4
	Leaky gut syndrome	1	4.4
	Faecal loading/compaction	2	8.6
	Candida	1	4.4
	Tape worm infection	1	4.4
	Total	23	100

Following from this, the study investigated how frequently the children receive treatment for intestinal parasites/worms. Most reported that their child gets treated once a year (41.1%; N = 81); 68 participants (34.5%) indicated twice a year; 15 (7.6%) said once every second year; and 26 (13.2%) answered that their child has never received treatment for parasites/worms. According to Giacometti et al. (1997), intestinal parasites/worms can disrupt the integrity of the gut lining and the health of the microbiome whilst increasing the risk for nutritional deficiencies and mental health problems. The possible impact of these gut pathogens on children's cognitive functioning are elicited through qualitative responses by parent participants: "*We started a parasite cleanse in April, and it feels as if my sons brain woke up"; "My son's stool was never normal until we treated the parasites and now daily use intestiflora. He started improving DRAMATICALLY after the problem was treated and his gut health kept in check. His mood, learning ability appetite etc. improved".*

Parent participants were then asked whether they notice a relationship between their child's ADHD and gut dysbiosis symptoms to which 61 (31%) responded "maybe" and 30 (15.2%) "yes". They were then given an open-ended question and asked to try to explain their related observations and to comment on any other relevant factors. This question elicited some important qualitative information, which have been grouped into themes and incorporated in the relevant sections throughout the paper. The rest will now be discussed briefly in relation to observations specifically related to the possible relationship between ADHD and gut dysbiosis symptoms.

First, several participants emphasised that constipation is a noticeable problem for their child: "*Battles with constipation frequently"; "Very constipated drink purgole daily to help."; "He stays in the toilet for long."; "She gets constipated & then just focusses on that."*. Chronic constipation is frequently a biomarker of gut dysbiosis (Mancabelli et al., 2017). Interestingly, one parent made the following comment which reveals the link between ADHD symptoms severity and constipation: "*ADHD symptoms worsen as well as behaviour deteriorates when constipates, also introceptive abilities deteriorate drastically when constipated."*

Second, this open-ended question elicited the theme of children often complaining of stomach aches: *"She complains of tummy ache every single day and I have always had this feeling that her gut is not quite right."*. In addition, it revealed a link between stomach aches/cramps, constipation and diarrhea/fecal incontinence, all of which have been associated with gut dysbiosis and found in another sample of children with ADHD (Ming et al., 2018): *"He gets stomach pains frequently and is often constipated, which has never been a problem for him."; "Some days he will complain about his tummy that hurts, and the stools that doesn't want to come out and then a few days later, he will run to the bathroom a few time during the day as if he cannot stop it*".

Furthermore, the association between stomach aches/cramps and anxiety/chronic stress which emerged in the responses furthermore suggests a bidirectional relationship between the brain and the gut via the gut-brain axis: *"Anxiety due to ADHD causes tummy ache."; "When worked up or bad day will complain of sore stomach."*. This serves to echo previous research findings related to the impact of stress on the gut microbiome and vice versa (Cong et al., 2015; Foster et al., 2017; Rakers et al., 2017; Rosin et al., 2020; van den Bergh et al., 2017; Vogel et al., 2020), alluding to the possible mediating role played by the gut microbiome in anxiety/stress and ADHD symptoms.

Finally, this question allowed parents the opportunity to comment on their general observations of the relationship between their child's ADHD symptoms and gut health: *"I've only linked the 2 now - she battles with her stomach, can't always control it and has excessive gas."; "He also has a lot of stomach complains plus the vomiting."; "We have had numerous tummy issues and have had to navigate those and have affected his mental health."*. From these various statements it is evident that there is sufficient reason to suspect a significant

relationship between ADHD and gut microbiome dysbiosis, although further investigation is required to generalize these findings.

Integration Summary

ADHD is the most prevalent neurodevelopmental disorder in the world, with an increasing number of children being affected by this disorder often proceeding into adulthood (Bakare, 2012; Meyer et al., 2004; Polanczyk et al., 2014; Simon et al., 2009; Thomas et al., 2015). ADHD is associated with a range of adverse outcomes and a staggering cost to health care systems, which may explain why this disorder has received so much international research attention (Cussen et al., 2012; Harpin, 2005; Hoza, 2007; Loe & Feldman, 2007; Nigg, 2013; Sharma & Couture, 2013). Despite many years of rigorous investigation, however, ADHD continues to portray a highly complex clinical picture, which have been attributed to aetiological heterogeneity (Cortese & Coghill, 2018; Nigg, 2012; Sciberras et al., 2017; Sharma & Couture, 2013; Silva et al., 2014; Tarver et al., 2014; Thapar et al., 2012; van Dyk et al., 2014).

Researchers have increasingly started implicating the role of the gut microbiome in ADHD and hold that the combination of an individual's genetics and unique microbial profile together with environmental risk factors could be indispensable for explaining ADHD development and the heterogeneity of symptomatic manifestations (Akram, 2017; Boonchooduang et al., 2020.; Bull-Larson & Mohajeri, 2019; Casas et al., 2019; Cenit et al., 2017; Dam et al., 2019; Lacorte et al., 2019; Mathee et al., 2020; Ming et al., 2018; Prehn-Kristensen et al., 2018; Sandgren & Brummer, 2018; Sukmajaya et al., 2021; Tognini, 2017; Wang et al., 2020). Research in this emerging field appears to have been predominantly conducted from a microbiological perspective, with an evident need for interdisciplinary research to provide a more comprehensive understanding from multiple perspectives. This study therefore aimed to provide a clinical psychological lens to the existing body of ADHD and gut dysbiosis research, offering a unique humanistic perspective to assist in bridging theoretical gaps, whilst placing the findings within the South African context.

A mixed methods research (MMR) approach with a quantitative dominant convergent design was deemed most appropriate to address this complex research objective in a comprehensive manner. The relationship between symptoms of gut dysbiosis and ADHD symptom severity was first explored from a quantitative perspective using the *Gastrointestinal Severity (GIS) Index* and the *ADHD Rating Scale-IV*, where a significant correlation was found

for the *Inattentive subscale* with the Pearson's product-moment correlation ($p = 0.026$). A multiple hierarchical regression analysis was then run to determine the variance between the *Inattention subscale* and independent variables related to gut dysbiosis, whilst controlling for possible confounding variables, related to gender, family ADHD heredity, and previously identified and possibly unrelated ADHD aetiological factors, which furthermore revealed a significant correlation ($p = 0.002$). The ANOVA analysis, which includes all confounding and all independent variables, also indicated a statistically significant effect on ADHD Inattention symptom severity ($p = 0.005$). Coefficients were then assessed, and the following four independent variables were found to make significant contributions to the prediction of Inattention symptoms severity ($p<0.05$) after all other possibly confounding variables were controlled for: *maternal prenatal smoking, mode of delivery, breastfeeding, and maternal prenatal obesity*. Following from this, the possible mediating role of gut dysbiosis with these independent variables was investigated through mediation analyses, although no significant mediation effect in terms of the *Gastrointestinal Severity (GIS) Index* scores was revealed.

These results were then further explored and expanded upon through a side-by-side comparison with the qualitative data obtained in a *self-report biographical questionnaire* in consideration of the perceived challenges in detecting gut dysbiosis in children with the *GIS Index* in isolation, whilst facilitating a more comprehensive understanding of the prominent aetiological risk factors involved.

First, it was found that most of the children in the study were born via C-section births, although the children who were born naturally scored higher on the ADHD Inattentive symptom severity subscale. This finding contradicted initial expectations based on previous research findings (Hoang et al., 2020; Nagpal & Yamashiro, 2017; Tribe et al., 2018); however, it is important to consider that many of these mothers reported birth complications, premature births and symptoms associated with gut dysbiosis which could have resulted in the infant inheriting a dysbiotic/imbalanced microbiome profile during birth (Talge et al., 2016).

Second, it was discovered that many of the children in the study struggle with a range of chronic health problems associated with low immunity and inflammation, such as skin conditions (i.e., eczema and rhinitis), allergies, sinusitis, and food intolerances, which have been correlated with both ADHD and gut dysbiosis (Camilleri, 2019; Feng et al., 2012; Hill et al., 2016; Leffa et al., 2018; Prince et al., 2015). This may also explain why so many of the children have been reported to receive regular antibiotic treatments, which is one of the main

risk factors for gut dysbiosis (Dinsmoor et al., 2005; Garcia-Gamboa et al., 2021; Rucklidge, 2013) and was found to have a statistically significant association with Inattentive symptom severity ($p < 0.05$). Following from this, it is worthy to note that gut dysbiosis symptoms are often subtle in presentation and therefore hard to detect, especially with child populations, and can be expressed indirectly in the form of consequential health problems, which are then frequently treated with antibiotics, exacerbating gut microbiome dysbiosis in the process (Bischoff, 2011; Ming et al., 2018).

Third, the study investigated children's diet and found that the children in this research sample have a daily intake of established gut irritants and microbiome *eubiosis* (i.e., balance) disrupters, the most prominent of which are processed sugar, artificial colourants, gluten, and dairy (Dam et al., 2019; Deans, 2016; Kan et al., 2018; Mathee et al., 2020). The possible impact of these foods on ADHD symptoms was accentuated by the qualitative reports from several parents. Conversely, the reported beneficial impact of probiotic supplements and a healthy diet on their child's ADHD symptoms furthermore adds emphasis to this relationship between ADHD and nutrition with the suspected mediating role of the gut microbiome (Kalenik et al., 2021; Pärrty et al., 2013; Stevens et al., 2019).

Fourth, the questions related to prenatal stress and early life stressors revealed that most of the mothers in the sample experienced significant prenatal stress, whilst many children were reported to have been exposed to a range of stressful early life events. In this regard, previous research studies have revealed chronic stress as a frequently over-looked gut microbiome disruptor, again indicating the possible mediating influence of gut dysbiosis in the established relationship between stress and ADHD (Cong et al., 2015; Foster et al., 2017; Rakers et al., 2017; Rosin et al., 2020; van den Bergh et al., 2017; Vogel et al., 2020). It is important to note, however, that this complex relationship also inherently contains a broad range of adverse psychosocial variants which could have significant implications for neurodevelopment and cognitive-, social- and behavioural functioning (Humphreys et al., 2018; Manyema & Richter, 2019; Nigg et al., 2020). This presents opportunities for further research to be conducted within the South African context where many children are exposed to increasingly stressful adverse life events (Manyema & Richter, 2019).

Fifth, open-ended questions related to gut health revealed that many children are struggling with other gastrointestinal symptoms not listed in the GIS Index (e.g., nausea), whilst several have been diagnosed with gastrointestinal disorders, such as irritable bowel

syndrome (IBS), spastic colon, leaky gut syndrome and a candida infection. Further, the question inquiring about worm/parasite treatment, which has been reported to impact mental health (Giacometti et al., 1997), revealed that some children displayed a noticeable improvement of ADHD symptoms following a cleanse. Following from this, participants were asked to comment directly on any observations related to a possible relationship observed between their child's ADHD symptoms and gut health. This open-ended question provided valuable insights into the bidirectional relationship of ADHD and the gut microbiome, along with the growing need for research in this emerging field.

Finally, through questions pertaining to current treatment strategies, it was established that most of the children in the study are currently taking ADHD medication and experiencing a range of adverse side-effects, the most prominent of which include a lack of appetite, insomnia, anxiety, mood- and behavioural problems, as well as symptoms associated with gut dysbiosis, such as stomach cramps and constipation. These reported observations confirm previous research findings (Hodgson et al., 2014; Purdie et al., 2002) and highlight the need for the development of alternative treatment options whilst necessitating a further investigation into the impact of the most commonly prescribed ADHD medication (i.e., Methylphenidate) in South Africa on the health of the gut microbiome.

In conclusion, the combination of these quantitative and qualitative results through mixed methods research (MMR) allowed for a more comprehensive conceptualisation to emerge on ADHD aetiology, both as a whole and in relation to the role of the gut microbiome. It provided a general overview of the overlap between the risk/protective factors involved in ADHD and gut dysbiosis, whilst elucidating the various bidirectional pathways involved in this intricate, emerging relationship between gut health and mental health.

Limitations and Recommendations

The gut-microbiome-brain connection has received increasing international research focus and findings have rapidly expanded our conceptualisation of mental health. According to a systematic review by Constantini et al. (2017), approximately 100 papers on the gut microbiome were published in 2007, whereas approximately 3000 were published in 2016, and the numbers are continuously increasing along with global research interest. It appears as though most studies have been conducted with adult populations, revealing the intricate nature of the subtle yet profound impact gut microbes have on mental health.

In recent years, however, there has been a surge in international research with children focusing on neurodevelopmental disorders and the gut microbiome. Since deciding on this research topic in the beginning of 2019, at least 23 studies on the link between ADHD and the gut microbiome have been published, when only four studies could be found at the time, and this is not even to mention the hundreds of studies published about ADHD in general during this short time period. The pace of this exponentially expanding area of research interest made for an exciting study yet presented a unique set of challenges as the literature needed to be constantly adapted to accommodate new findings.

The interdisciplinary nature of this study necessitated a firm theoretical foundation in basic gut microbiology and the neurophysiology of ADHD, which was, on the outset, beyond the researcher's scope of formal education. Thus, a co-supervisor from the microbiology department was included in the research team who could provide the required expertise, and the research supervisor from the psychology department enabled a firm foundation in psychological theory and research methodology.

In light of the shortcomings of existing gut dysbiosis measurement questionnaires along with the need for a more humanistic focus, a mixed methods research (MMR) approach was deemed most appropriate. Mixed methods studies are generally more challenging to implement than a quantitative or qualitative study as they require the researcher to have sufficient knowledge and experience in both approaches with the ability to integrate the two different data sources in a coherent manner (Creswell & Creswell, 2018). MMR can also be more labour intensive and time consuming in terms of the planning and qualitative data analysis, especially when a large sample population is used to attain statistical significance (Creswell & Tashakkori, 2007). It was consequently decided to focus on quantitative data and to limit the qualitative data to only a few open-ended questions to help explain and expand on the statistical results. The qualitative data provided important information, which could not have been elicited through a quantitative inquiry in isolation, and it served to enhance the validity of the findings through triangulation.

Findings could be deemed relatively generalisable across different cultures and contexts due to the common physiological mechanisms involved in both ADHD and gut microbiome dysbiosis. Due to the transparency of the research process and availability of the online survey, other researchers would also be able to replicate the study with relative ease. This research furthermore has the potential to serve as a pilot study through which

participants can be identified for future interdisciplinary research, which could expand on qualitative data through semi-structured interviews to obtain a more in depth understanding of the various psychosocial factors involved. Alternatively, researchers could focus on developing an experimental study through which dietary and/or probiotic interventions could be refined for this specific subgroup within the ADHD population.

Even though the mixed methods approach presented unique challenges and extended the time required to complete this study, it facilitated a broader perspective of the various factors involved and revealed the biopsychosocial complexity of ADHD as a multidimensional disorder. Through this research process and in consideration of the implications of these findings, the need for more interdisciplinary research became evident to make progress towards addressing the most prolific neurodevelopmental disorder globally in a more integrated and efficient manner.

Shifts away from traditional lifestyles and diets rich in cultured foods towards increasingly sterile internal and external environments are known to impact gut microbiome compositions and functioning, thereby increasing the risk of neurodevelopmental disorders, such as ADHD (Cenit et al., 2017). Modernization has furthermore resulted in an increasing number of women undergoing caesarean sections, which has shown to impact the composition of the commensal microbiome (Checa-Ros et al., 2021). Finally, in light of the current international COVID-19 pandemic, children are increasingly being raised in more sterile environments, restricting exposure to microbes and thus the maturation of a child's microbiome (Burchill et al., 2021; Kim et al., 2019).

Conclusion

This study aimed to facilitate more comprehensive conceptualisations of ADHD aetiology and the various risk/protective factors involved, which could serve to refine preventative and treatment strategies, shifting the focus from genetic heredity, and presenting neurological symptoms towards protecting and enhancing the health of the gut microbiome. Quantitative and qualitative findings from this study converged in support of the hypothesis that the gut microbiome may play a significant role in neurodevelopment and cognitive functioning in children diagnosed with ADHD. A healthy gut microbiome early in life may therefore act as a key protective factor against ADHD and other neurodevelopmental disorders, whilst the amelioration of gut dysbiosis as an effective treatment option for ADHD will need to be investigated in future research studies.

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Appendix A: Questionnaire Booklet

Appendix A1: Self-compiled biographical questionnaire

Please complete the next set of questions by marking the appropriate choice with an X or writing the answer in the space provided.

1.1. Child Biographical Information:

1.1.1. Date of birth: _____

1.1.2. Gender: _____

1.1.3. Ethnicity_____

1.1.4. Home language: _____

1.1.5. Language of education: _____

1.2. Family ADHD:

1.2.1 Has one or both parents ever been diagnosed with ADHD? *YES / NO*

1.2.2. Have one or more siblings ever been diagnosed with ADHD? *YES / NO*

1.3. Psychological & Medical Diagnostic History

1.3.1. Has your child ever been diagnosed with ADHD by a professional medical/mental health practitioner? *YES / NO*

1.3.2. By whom was your child diagnosed? (i.e., psychologist/psychiatrist/paediatrician/general practitioner/other)

1.3.3. At what age was your child diagnosed? _____

1.3.4. Is your child currently taking any prescribed medication? *YES / NO*

1.3.5. Name of prescribed medication (if any): _____

1.3.6. Please briefly describe the side-effects of the ADHD medication (if any):

_____

1.3.7. Please specify if your child has ever been diagnosed with a psychological disorder (apart from ADHD)? (e.g., anxiety, depression, encopresis, enuresis; autism spectrum disorder; specific learning disorder; intellectual disability; oppositional defiant disorder; etc.)

_____

1.3.8. Please specify if your child has any recurrent or chronic health conditions: (e.g., epilepsy, traumatic brain injury; thyroid dysfunction; eczema; allergies; sinusitis; flu)

_____

1.3.9. Did your child ever have colic? *YES / NO*

1.3.10. Did your child take any antibiotics during the first 3 years of life? *YES / NO*

1.3.11. On average, how many courses of antibiotics does your child take per year?

_____

1.3.12. Has your child ever been exposed to environmental toxins? (e.g. heavy metals) *YES / NO*

1.3.13. If yes, please specify: _____

1.4. Developmental History:

Please rate the frequency of the following:

1.4.1. How frequently did the biological mother of the child use tobacco during pregnancy?

0 (never) *1 (seldom)* *2 (often)*

1.4.2. How frequently did the biological mother use alcohol during pregnancy?

0 (never) *1 (seldom)* *2 (often)*

1.4.3. How frequently did the biological mother use antibiotics during pregnancy?

0 (never) *1 (seldom)* *2 (often)*

1.4.4. How frequently did the biological mother experience abnormally intense stress during the pregnancy?

0 (never) *1 (seldom)* *2 (often)*

1.4.5. Please specify if the biological mother struggled with any health problems during pregnancy (e.g., urinary tract infection, constipation, etc.):

_____

1.4.6. Please indicate whether the biological mother was overweight during the pregnancy:

0 (not at all) *1 (mildly overweight)* *2 (overweight)*

1.4.7. Was your child born prematurely? *YES / NO*

1.4.8. Please specify the pregnancy period in weeks (if possible):_____

1.4.9. Please specify the birth weight of the child (if possible):_____

1.4.10. Mode of delivery: *C-Section / Natural birth*

1.4.11.1. Were there any labour complications? *YES / NO*

1.4.11.2. If yes, please specify the nature of the labour complications:

_____

1.5. Early Life Nutrition:

1.5.1. Was your child breastfed? *YES / NO*

1.5.2. If yes, please indicate the breastfeeding period in weeks (if possible):

_____

1.5.3. Did your child drink formula milk? *YES / NO*

1.5.4. If yes, please indicate the formula milk period (if possible): _____

1.5.5. Please specify if your child takes any daily supplements (i.e., vitamins, minerals, Omega 3, probiotics, etc.):

_____

1.5.6. ***Please rate how often your child consumes the following:***

1.5.6.1. Processed sugar & artificial colourants (i.e., sweets, cooldrink, etc.)

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.6.2. Gluten (i.e., bread, pasta, cakes, etc.)

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.6.3. Dairy (i.e., milk, cheese, etc.)

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.6.4. Caffeine (i.e., coffee, energy drinks, etc.)

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.6.5. MSG (i.e., potato chips, Aromat, etc.)

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.6.6. Raw fruit (i.e., apples, bananas, oranges, etc.)

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.6.7. Raw vegetables (i.e., carrots, lettuce, etc.)

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.6.8. Water

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.7. How healthy do you perceive your child's general diet to be on a scale from 1 to 5?

1.5.8. Is your child a "picky eater"?

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.9. Does your child ever struggle with his/her appetite?

0 (Never) 1 (Rarely) 2 (Sometimes) 3 (Often) 4 (Daily)

1.5.10. Please specify if your child has any food allergies:

_____

1.6 <u>Early Life Stress:</u>

1.6.1. During the first 3 years of your child's life, how frequently was the mother and infant separated for significant periods of time?

0 (never) 1 (seldom) 2 (often)

1.6.2. During the first 3 years of your child's life, how often did the mother struggle with significant post-natal depression?

0 (never) 1 (seldom) 2 (often)

1.6.3. During the first 3 years of your child's life, how frequently was he/she exposed to significant family conflict?

0 (never) 1 (seldom) 2 (often)

1.6.4. During the first 3 years of your child's life, how frequently was he/she exposed to any other significantly stressful life events (i.e., divorce, death of a loved one, financial stress, home relocation, etc.)?

0 (never) 1 (seldom) 2 (often)

1.6.5. During the first 3 years of your child's life, how frequently was he/she hospitalized?

0 (never) 1 (seldom) 2 (often)

Appendix A2: ADHD Rating Scale given to parents to complete as a means of gathering ADHD symptom severity scores for their child

Circle the number that best describes your child's behaviour over the past 6 months (when he/she is NOT on medication).	**Never or Rarely**	**Some-times**	**Often**	**Very Often**
2.1. Fails to give close attention to details or makes careless mistakes in schoolwork.	**0**	**1**	**2**	**3**
2.2. Fidgets with hands or feet or squirms in seat.	**0**	**1**	**2**	**3**
2.3. Has difficulty sustaining attention in tasks or play activities.	**0**	**1**	**2**	**3**
2.4. Leaves seat in classroom or in other situations in which remaining seated is expected.	**0**	**1**	**2**	**3**
2.5. Does not seem to listen when spoken to directly.	**0**	**1**	**2**	**3**
2.6. Runs about or climbs excessively in situations in which it is inappropriate.	**0**	**1**	**2**	**3**
2.7. Does not follow through on instructions and fails to finish work.	**0**	**1**	**2**	**3**
2.8. Has difficulty playing or engaging in leisure activities quietly.	**0**	**1**	**2**	**3**
2.9. Has difficulty organizing tasks and activities.	**0**	**1**	**2**	**3**
2.10. Is "on the go" or acts as if "driven by a motor."	**0**	**1**	**2**	**3**
2.11. Avoids tasks (e.g., schoolwork, homework) that require sustained mental effort.	**0**	**1**	**2**	**3**
2.12. Talks excessively.	**0**	**1**	**2**	**3**
2.13. Loses things necessary for tasks or activities.	**0**	**1**	**2**	**3**
2.14. Blurts out answers before questions have been completed.	**0**	**1**	**2**	**3**
2.15. Is easily distracted.	**0**	**1**	**2**	**3**
2.16. Has difficulty awaiting turn.	**0**	**1**	**2**	**3**
2.17. Is forgetful in daily activities.	**0**	**1**	**2**	**3**
2.18. Interrupts or intrudes on others.	**0**	**1**	**2**	**3**
Total Score				

Appendix A3: The Gastrointestinal Severity (GIS) Index given to parents to assess gut microbiome dysbiosis symptom type and severity

GASTROINTESTINAL SEVERITY (GIS) INDEX		
Please indicate how often your child experiences the following:		Score
3.1. Constipation	0 = 5 or more stools/week 1 = 3-4 stools/week 2 = 0-2 stools/week	= _____
3.2. Diarrhea	0 = 0-1 loose stools/day 1 = 2-3 loose stools/day 2 = 4 or more loose stools/day	= _____
3.3. Average Stool Consistency	0 = Formed 1 = Loose/unformed 3 or more days/week 2 = Watery 3 or more days/week	= _____
3.4. Stool Smell	0 = Normal 1 = Abnormal 3 or more days/week 2 = Unusually foul	= _____
3.5. Flatulence	0 = Normal 1 = Frequent 3 or more times/week 2 = Daily	= _____
3.6. Abdominal Pain	0 = None 1 = Mild discomfort 3 or more times/week 2 = Moderate to severe discomfort 3 or more times/week	= _____
Total Score		= _____

3.7. Please specify if any other gastrointestinal (gut) symptoms are present (e.g., nausea, vomiting, indigestion, faecal incontinence):

_____

3.8. Please specify if your child has ever been diagnosed with a gastrointestinal condition? (e.g., Irritable Bowel Syndrome/Spastic Colon, etc.).

_____

3.9. How frequently does your child receive treatment for worms/parasites?

_____

3.10. Do you notice a relationship between your child's ADHD and gut symptoms?
YES / NO

_____

3.11. If yes, please try to explain your observations and comment on any other factors which may be important for us to know:

_____

Appendix B: Research Study Information Leaflet and Consent Form

INFORMATION LEAFLET

DATE: September 2020 – August 2021

PRINCIPLE INVESTIGATOR / RESEARCHER NAME AND CONTACT NUMBER:

Katrien van Zyl 2018505384 0613594062

FACULTY AND DEPARTMENT:

Faculty of the Humanities

Department of Psychology

STUDY LEADER(S) NAME AND CONTACT NUMBER:

Mr. Henry Taylor 051 401 9322 taylorhw@ufs.ac.za

TITLE OF THE RESEARCH PROJECT

Attention-deficit/hyperactivity disorder (ADHD) symptom severity and gut microbiome dysbiosis: An aetiological perspective on an emerging relationship.

This study is the first of its kind in South Africa and aims to investigate the link between ADHD symptoms and gut microbiome dysbiosis symptoms (i.e., digestive problems) with a specific focus on the various factors involved. Due to COVID-19 restrictions, this study will be conducted online, allowing you to participate from a safe location of your choice and at a time that is convenient for you. The questionnaire should only take approximately 15min to complete, after which nothing else will be required from you.

You are kindly being invited to participate in this study on behalf of your child as one of approximately 200 parents of children between the age of 6 and 12 who have been diagnosed with ADHD. Your participation in the study will contribute to the growing body of ADHD research in South Africa through which more effective prevention and treatment strategies can be developed.

Your child will form the focus of this study, although you will participate on his/her behalf due to your awareness and objectivity regarding your child's developmental history and his/her symptoms. Please explain the study to your child in simple terms and make sure he/she agrees for you to answer the questions, ensuring that there will be absolutely no negative repercussions if he/she decides to decline the opportunity.

Please note that participation is completely voluntary and you are free to withdraw at any time. It is not foreseen that there are any risks associated with your participation for you or your child and all information gathered will be kept strictly confidential on a password protected laptop. The data will only be accessibly to members of the research team and will be used for the purposes of a Clinical Psychology master's dissertation, and possibly for journal articles or conference presentations in the future.

This study has received full approval from the Research Ethics Committee of the Faculty of the Humanities of the University of the Free State (UFS-HSD2020/1384/0411). If you have any concerns about the way in which the research is conducted, you may contact the administrator of the Faculty of Humanities Research Ethics Committee, Mrs. Charné Vercueil, on VercueilCC@ufs.ac.za or 0514017083. Furthermore, should any distress arise as a result of participation in this study, Mr. Taylor, the coordinator of the Adult Practice of the UFS Department of Psychology, may be contacted (051 401 9322) for 2-3 psychotherapeutic sessions as deemed necessary at the initial consultation and the service will be provided to you free of charge. You can also contact MobieG, an online counselling platform which renders their services free of charge by sending a WhatsApp to 0637043030 or an email to ops@mobieg.co.za.

If you would like to be informed about the final research findings or if you have any questions, please contact the researcher, Katrien van Zyl, on 061 359 4062 / adhdgutconnection@gmail.com. Should you request feedback on the final results of this study, you may benefit in terms of enhanced knowledge on the possible relationship between the severity of ADHD symptoms and gut dysbiosis symptoms. Due to the anonymous nature of the study, it would, however, not be possible to provide specific feedback on your personal results.

Thank you in advance for your helpful contribution to ADHD research and for assisting me with this exciting study as part of the completion of my master's degree in Clinical Psychology. Your participation is sincerely appreciated!

INFORMED CONSENT AGREEMENT

Please take note of the following informed consent agreements and indicate a "yes" in the tick box below to continue with the questionnaire:

I have read and understood the study as explained in the information sheet, which contains sufficient information regarding the nature, procedure, potential benefits, and anticipated inconvenience of participation in the study.

I understand that my participation is completely voluntary and that I am free to withdraw at any time without penalty. I am aware that the findings of this study will be anonymously processed into a master's dissertation, journal publications and/or conference proceedings and under no circumstances will my name or identifying characteristics be included in any documentation.

I agree to complete the self-report questionnaires (*ADHD Rating Scale IV, Gastrointestinal Severity (GIS) Index and a self-report biographical questionnaire*) on behalf of my child.

I have explained the study to my child, and he/she understands that participation is completely voluntary and that we can withdraw at any time. My child agrees to let me answer the questions on his/her behalf.

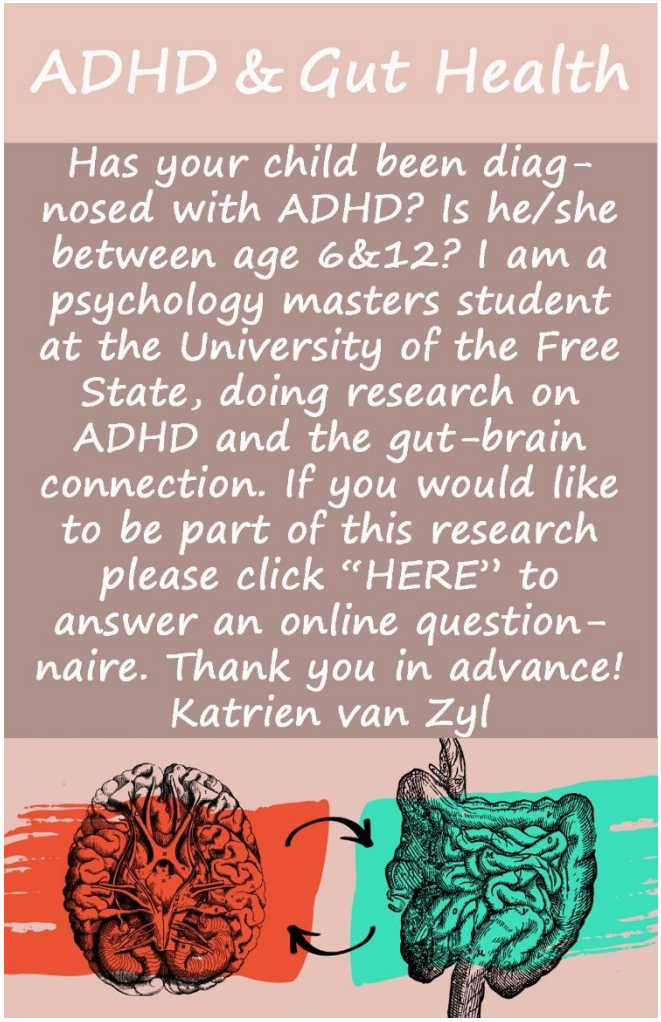
I understand that the data from the questionnaires will be treated as strictly confidential and will be available only to members of the research team. I understand that should my child or I suffer any distress due to the process of participation in this study, we are both eligible for psychological counselling and may contact Mr. Taylor who will arrange 2-3 counselling sessions, as deemed necessary, free of charge. In addition, should I have any concerns about the way in which the research has been conducted I may contact the administrator of the General Human Research Ethics Committee of the UFS, Mrs. Charné Vercueil on vercuilcc@ufs.ac.za or (051) 4017083.

I know that I can contact the researcher on 061 359 4062/adhdgutconnection@gmail.com should I have any questions regarding the research.

By submitting my anonymous information, I am giving my consent to participate in this study on behalf of my child who agrees for me to do so.

Thank you sincerely much for your willingness to participate in this study!

Appendix C: Research Invitation



Appendix D: Qualitative Data

Q.	Themes	Subthemes	Responses
1.3.5.	Type of medication (TM)	Ritalin	104
		Concerta	28
		Neucon	28
		Risperdal	23
		INIR	9
		Contramyl	9
		Strattera	6
		Laxatives	4
		Lorien	3
		Citalopram	3
		Melatonin	2
		Epilim	1
		Ethipramine (Tofril)	1
		Abilify	1
		Circadin	1
		Lexamil	1
		Tripeline	1
		Stresam	1
		Serdep	1
1.3.6.	Side effects of ADHD Medication (SEM)	Decreased appetite	85
		Insomnia	27
		Emotional/mood-swings	15
		Stomach cramps	14
		Anger/aggression/irritation	14
		Anxiety	10
		Apathy/withdrawn/spacey	9
		Weight gain	8
		Depression	7
		Headaches	7
		Drowsiness/lethargy	6
		Increased appetite	5
		Nausea/vomiting	4
		Weight loss	3
		Tics (vocal & physical)	3
		Talkative	2
		Dry lips/mouth	2
		Behavioural problems	2
		Skin rash	1
1.3.7.	Psychiatric Comorbidities (PsyC)	Anxiety	46
		ODD	15
		Autism & Aspergers	13
		Sensory processing disorder	11
		Depression	9
		Learning disorder	5
		Bipolar / Emotional instability	4
		Dyslexia	3
		Dyspraxia	3
		Apraxia	2
		Anger outbursts	2
		Intellectual disability	2
		Separation disorder	1

		Speech impairment Aggression PTSD	1 1 1
1.3.8.	Physical Comorbidities (PhyC)	Sinusitis & postnasal drip Allergies Eczema Asthma Chronic constipation Ear infections (Allergic) Rhinitis Epilepsy Gluten/lactose intolerance Febrile convulsions/seizures Brain injury Irritable Bowel Syndrome RSV Reflux Depressed immune system Optic neuritis	34 26 17 14 5 4 3 3 3 2 1 1 1 1 1 1
1.3.11.	Average courses of antibiotics per year (AC)	- None: 12 - 0 – 1: 25 - 1 – 2: 16 - 2 – 3: 8 - 3 – 4: 4 - 4 – 5: 4 - 5<: 6	*- Now barely any but as child monthly from 12 to 26 months of age due to tonsillitis* *- Mostly he was feverish so was always given antibiotics* *- From birth to 5 years, +/-3 courses per year. 5 years to current, none.* *- Between ages 0-3 at least 4 doses per year* *- From birth to age 6, approximately 4 to 5 times a year. From age 6 to 9, once or twice a year* *- Till age of 6 she was sick on very regular basis; antibiotics was given no less than 4 to 6 times a year. After tongsils and adinoids and grommets were done at age 3 improvement started but very easily picked up infections and common cold* *- When he was a baby about 3. After his tonsils was removed, almost 0. He is now 10 and has not had antibiotics for the last 3 years. He takes a gut microbe supplement every day.* *- Up until the age of 6, at least 4-5 times a year* *- First 4 years at least 3 times per year* *- Maybe once a year after tonsils were removed before tha about 3 times per year.* *-Up to 3 years of age, he had alot of antibiotics almost every second month.*

			- Up to age 8 plenty thereafter about twce a year this year only onceice per year this year no *- Hardly any now but as a baby he was sick all the time. Had to have his tonsils & adenoids out. Grommets.* *- It used to be 2 to 3 a year when he was younger but hasnt had any for the last 3 to 4 years. We all have the flu vaccine.* *- Now, about zero. Between the ages of 1.5 and 3 - around 2 courses every 5 months due to reoccurring tonsillitis. We assume that this is the reason for her constipation.* *- Now maybe only 1, when she was younger frequently due to ear infections.* *- More than 5* *- 6 times* *- 6 times* *- Many due to the eczema* *- No idea. Adopted but what I gather a lot due to him not being treated correctly for ear infection etc.*
1.5.5.	Daily supplements (DS)	Multivitamin Omega 3 Zinc Iron Magnesium Vit C Vit B Vit D	50 46 23 4 6 12 5 2
3.7.	Other gastrointestinal symptoms (OGS)	Nausea Vomiting Faecal incontinence Indigestion Excessive burping Frequent defaecation Heartburn Acidity	18 8 4 3 1 1 1 1
3.8	Gastrointestinal conditions formally diagnosed (CFD)	Irritable Bowel Syndrome Encopresis Spastic colon Chronic constipation Reflux Gut inflammation Leaky gut syndrome Faecal loading/compaction Candida Tape worm infection	5 4 3 2 3 1 1 2 1 1

5.10.2.	If you notice a relationship between your child's ADHD and gut symptoms, please try to explain your observations and comment on any other factors which may be important for us to know. Observations (O)	Stomach aches/cramps (OS)	*- Anxiety due to ADHD causes tummy ache.* *- When worked up or bad day will complain of sore stomach.* *- He gets stomach pains frequently and is often constipated, which has never been a problem for him.* *- Some days he will complain about his tummy that hurts, and the stools that doesn't want to come out and then a few days later, he will run to the bathroom a few time during the day as if he cannot stop it.* *- Hannah complains of tummy ache every single day and I have always had this feeling that her gut is not quite right.* *- When taking meds he gets stomach cramps and discomfort*
		Constipation (OC)	*- ADHD symptoms worsen as well as behaviour deteriorates when constipates, also introceptive abilities deteriorate drastically when constipated.* *- Well, she doesnt eat much. which is causing her to be constipated, even before taking medication she was always complaining about her stomach.* *- Very constipated drink purgole daily to help.* *- He stays in the toilet for long.* *- Battles with constipation frequently* *- She gets constipated & then just focusses on that.*
		Parasites/worms (OPW)	*- We started a parasite cleanse in April, and it feels as if my sons brain woke up.* *- My son's stool was never normal untill we treated the paracites and now daily use intestiflora. He started improving DRAMATICALLY after the problem was treated and his gut health kept in check. His mood, learning ability appetite etc. improved.*
		Benefits of probiotics (OBP)	*- Before medicine for ADHD, he was constipated and picky eater. When he was 5 I gave him probiotics regularly and I feel that definitely improved the bowels. I would have carried on, but expenses are a factor (cost of probiotic supplements).*

			- I hugely believe in the gut being linked to mental health. I need to have her on a probiotic more consistently to give a better evaluation. *- Probiotics do him good* *- He seems to be a little bit more calm when consistently taking probiotics.*
		Impact of diet (OID)	*- I have decreased gluten and sugar hugely and have found that she complains less about thrush.* *- Sugar causes major irritability, frustration and affects concentration.* *- We have changed our diet by cutting out gluten and dairy on top of sugar. He seems less impulsive and more cooperative. There has also been no incidents at school the last two weeks. Whereas previously, there were at least two a week.* *- It is difficult to quantify as I know his diet is not conducive to good gut health. That is why I insist on the microflora supplement.* *- I realised this a year ago. Unhealthy diet... high carbs and high sugar intake made him less focussed and more irritable. We changed our whole family's diet and he has also been off all medication for 10 months now, just by changing his diet.* *- He seems more hyper and struggles to focus when he eats gluten and sugar.* *- Concentration and any form of schoolwork and emotional instability is worse when she has eaten sugar, milk, wheat or gluten.* *- Dairy, colourants additives and preservatives make things worse.* *- Hyper from fizzy drinks*
		General digestive issues (OGDI)	*- I've only linked the 2 now - she battles with her stomach, can't always control it and has excessive gas.* *- We have had numerous tummy issues and have had to navigate those and have affected his mental health.* *- He also has a lot of stomach complains plus the vomiting.* *- Had issues with underdeveloped nerves in his intestine and had to have surgery at a year old for it.*

Appendix E: Ethical Clearance Letter

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

GENERAL/HUMAN RESEARCH ETHICS COMMITTEE (GHREC)

02-Nov-2020

Dear Ms Van Zyl, Katrien K

Conditionally Approved

Research Project Title:
Attention-deficit/hyperactivity disorder (ADHD) symptom severity and gut microbiome dysbiosis: An aetiological perspective on an emerging relationship

With reference to your application for ethical clearance for your research: it has been determined by the General/Human Research Ethics Committee of the University of the Free State that this research is ethically sound and may receive full ethical approval after the following provision(s) have been attended to:

Outcome: Conditional approval.

The application complies with the required ethical standards. Conditional approval should be granted pending the written approval from ADHASA to assist in the study and provide access to their client database.

Please ensure that you comply with all government and UFS protocols related to COVID-19 when conducting research.

Please attend to the abovementioned within sixty (60) days. Failure to respond or make prior arrangements within this time will result in your application being withdrawn (terminated). Please note: **This is not a valid ethical approval until you (the applicant) have attended to the above mentioned provisions and the ethics committee has validated them.**

Yours sincerely

Dr Adri Du Plessis
Chairperson: General/Human Research Ethics Committee

205 Nelson Mandela Drive
Park West
Bloemfontein 9301
South Africa

P.O. Box 339
Bloemfontein 9300
Tel: +27 (0)51 401 9337
duplessisA@ufs.ac.za
www.ufs.ac.za

Appendix F: Permission to Submit

Reference: Mr HW Taylor
Psychology Building Room 204
University of the Free State
BLOEMFONTEIN
South Africa
Telephone: 051 401 9322
Email: taylorhw@ufs.ac.za

July 2021

PERMISSION TO SUBMIT

Student: Katrien van Zyl

Degree: Masters of Social Sciences (Clinical Psychology)

Department: Psychology

Title: Attention-Deficit/Hyperactivity disorder (ADHD) symptom severity and gut microbiome dysbiosis: an aetiological perspective on an emerging relationship

We hereby provide permission that this dissertation be submitted for examination – in partial fulfilment of the requirements for a Master's in Psychology, in the Department of Psychology, Faculty of the Humanities, at the University of the Free State.

We approve the submission for assessment and that the submitted work has not previously, either in part or in its entirety, been submitted to the examiners or moderators.

Kind regards,

Mr. HW Taylor
Supervisor

Dr E Cason
Co-Supervisor

Appendix G: Proof of Language Editing

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

Centre for Teaching and Learning / Onderrig-en-Leersentrum

13 July 2021

To whom it may concern,

I, Gawain Norval, hereby declare that I have proofread the master's dissertation entitled, *ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD) SYMPTOM SEVERITY AND GUT MICROBIOME DYSBIOSIS: AN AETIOLOGICAL PERSPECTIVE ON AN EMERGING RELATIONSHIP*, by Katrien van Zyl.

I am employed at the Centre for Teaching and Learning (CTL), as a Facilitator of Academic Literacy within the Unit for Academic Literacy and Language Development (ALLD) at the University of the Free State (UFS). Due to my experience and expertise teaching Academic Literacy, I am intimately familiar with the language, grammar, and organisational requirements of an academic thesis and, therefore, qualified to proofread this document.

Please feel free to contact me should you require further information regarding the proofreading of this document, either telephonically, at 083 681 1546, or per email, at NorvalGT@ufs.ac.za.

Yours faithfully,

Mr Gawain Norval
Centre for Teaching and Learning
Literacy Assistant: Unit for Academic Literacy and Language Development

Appendix H: Turnitin Report

Research Dissertation

ORIGINALITY REPORT

16%	10%	8%	9%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of the Free State Student Paper	4%
2	Thais Martins-Silva, Angélica Salatino-Oliveira, Júlia Pasqualini Genro, Fernando D.T. Meyer et al. "Host genetics influences the relationship between the gut microbiome and psychiatric disorders", Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2020 Publication	<1%
3	scholar.ufs.ac.za Internet Source	<1%
4	ecommons.luc.edu Internet Source	<1%
5	www.coursehero.com Internet Source	<1%
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