Gastrointestinal symptoms in autistic children: their impact, experience of healthcare and the efficacy of dietary interventions

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I, Susan Simmons confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. This work has been submitted through Turnitin and the only similarities found were to my previously published work.

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Abstract

A randomised double-blind placebo-controlled crossover trial of Vivomixx probiotic was conducted for autistic children aged 3-15 years with at least one persistent gastrointestinal symptom. Vivomixx was not found to improve global function or reduce the frequency of gastrointestinal symptoms in this heterogeneous group. A subgroup of participants displayed a notable improvement in global function following treatment with Vivomixx but post-hoc correlation analysis did not isolate any characteristics that identified these from the rest of the participants.

The experience of autistic children with gastrointestinal symptoms, the impact of those symptoms and their experience of related healthcare, has not been explored in the UK. Twelve in-depth interviews were conducted with parents of autistic children with persistent gastrointestinal symptoms. These explored the impact of gastrointestinal symptoms on their autistic child and their family; day-to-day management of their child's gastrointestinal symptoms; their experience of related healthcare; and their opinions on participation in research studies. Seven major themes emerged from the analysis: i) Gastrointestinal symptoms impact on many aspects of the lives of autistic children and their families and the impact tends to increase with age; ii) Understanding the nature and severity of gastrointestinal symptoms in autistic children is complex and multifactorial; iii) Access to healthcare services for autistic children with gastrointestinal symptoms is variable and often limited, with diagnostic overshadowing; iv) Reasonable adjustments to the current NHS service are needed to reduce child and parent stress; v) Covid-19 lockdown and Covid-safe measures in schools affected gastrointestinal symptoms in autistic children but not in a uniform fashion; vi) There are barriers to involvement in a clinical trial for autistic children and their parents; vii) Parents' experience of participating in the VIVO-ASD clinical trial can help inform future autism gastrointestinal research study design.

Impact Statement

This research is unusual in documenting the significant impact of gastrointestinal symptoms on autistic children and their families. There has been very limited research into this area globally and to my knowledge is the first research of its kind in England. The findings extend the understanding of how multiple inter-related factors can contribute to expression of gastrointestinal symptoms in autistic children. It also highlights the difficulties that autistic children and their families face when trying to access health services and presents parents' suggestions for reasonable adjustments to the current National Health Service (NHS) healthcare offering. As a whole, my findings represent a unique and valuable resource to influence the development of guidelines for early management of gastrointestinal symptoms in autistic children. This, combined with the recommendations to improve the experience and accessibility of gastrointestinal healthcare services for this patient group should be a valuable resource for health policy makers and gastroenterology specialists. The findings as a whole should be a useful tool for clinicians without specific knowledge in autism, as an aid to embracing the complexity of this patient population without underestimating the possibility and value of symptom improvement. The findings regarding reasonable adjustments and the unusual presentation of gastrointestinal symptoms in autistic children should be useful to autistic children and young people and their families and care-givers.

The results of this qualitative research have already been presented to the lay public and to researchers and healthcare professionals with a special interest in autism as part of the 2022 Autistica Research Festival.

The results of the Vivo-ASD probiotic clinical trial for autistic children with gastrointestinal symptoms adds to the evidence regarding probiotic use in this group. Few studies have been conducted in this area and most involve a small number of participants. As a crossover study with 69 enrolled participants, it is the largest autism probiotic study yet conducted to my knowledge. The results confirm the safety of Vivomixx in this patient group. As a randomised double-blind placebo-controlled trial, a significant treatment effect was not found in the group as a whole, which is contrary to published open studies and this highlights the importance of robust design for future

studies. The identification of a subgroup that experienced a notable improvement in global function after Vivomixx treatment, suggests that further research with a more tightly defined subgroup may show positive results. Given the low risk of probiotic intervention and the high prevalence of gastrointestinal symptoms in autistic children, this warrants further research. The results of the qualitative interviews provide valuable insight into parents' views on the acceptability and practicality of various biologic and other outcome measures in autism gastrointestinal research. This provides an insight for clinicians on the challenges these families face with biologic tests and daily symptom recording for their autistic child. Not precluding the need to co-produce studies with autistic individuals and their advocates, this resource could inform the initial design of future autism intervention research studies enabling them to be more accessible and acceptable to families with an autistic child.

Table of contents

Table of tables

Table of figures

Chapter 1   Introduction

It is plausible that a relationship exists between the gut microbiome and some associated health issues commonly found in autistic children. An imbalance in the gut microbiota and associated health effects, may also promote certain behaviours associated with autism. Gastrointestinal (GI) symptoms occur frequently in autistic children [1] and research suggests that the impact of autistic traits is related to the severity of gastrointestinal symptoms [2][3]. However, current treatments are limited and not well evaluated.

This is a mixed methods research project. The first part used quantitative research to explore whether dietary intervention could help improve the overall function and GI symptoms of autistic children. The second part uses qualitative research to explore the impact and experience of gastrointestinal symptoms on autistic children and their families, and their experience of related healthcare and research.

1.1   Reasons behind hypothesis generation

The generation of this hypothesis is born out of my own clinical experience practicing personalised dietary intervention with autistic children. Having seen over two hundred autistic children while working as part of a multidisciplinary team in a private Environmental Medicine clinic, a pattern of unexpected response to dietary intervention was noted by myself and my colleagues (an Environmental physician and a Paediatric consultant). The clinic treated both adults and children and was not a specialist autism clinic. Patients had a variety of existing diagnoses including autism, attention deficit hyperactivity disorder (ADHD), Downs Syndrome, Chronic fatigue syndrome, Irritable Bowel Syndrome (IBS), allergies, anxiety and sleep disorders, however this research focusses on my autistic patients. Frequently the reason for autistic children coming to the practice was for digestive problems, gastrointestinal symptoms or concerns about limited diet and inadequacy of nutrients. We observed that when bowel movements were normalised and nutrient status improved, the children would improve in other aspects such as eye contact, interaction with siblings and parents, sleep, skin health, pallor, and mood. These improvements also reduced parental stress and put the child in a better position to learn. An analysis of data from this clinic is presented in Chapter 2.

1.2 Autistic Spectrum Disorder

Autism Spectrum Disorder (ASD) is a medical diagnosis based on observation and medical history taking. Some people with a diagnosis of ASD feel that it is a natural variation in neurology that should be accommodated for by reasonable adjustments in society and acceptance of difference. A recent systematic review calculated the median global prevalence of ASD at 65 per 10,000 with increasing prevalence over time [4].

Autism Spectrum Disorder is characterised by difficulties in social communication and understanding, and a rigidity of interests [5]. There is a wide difference in the impact of autistic traits from one person to another. Altered sensory responses are common [6] and are now considered part of the diagnostic criteria and core autism traits [7]. Social situations present challenges for autistic individuals and busy places can be overwhelming and can trigger sensory overload.

The latest UK prevalence estimate for autism in children is one in 57 which equates to 1.76% [8]. Of these, 18.1% also have a learning difficulty. The prevalence rate differs for boys (2.8%) and girls (0.65%). The prevalence rate also differs with the child's ethnicity, with the highest prevalence for those of black ethnicity (2.1%). The effect of ethnicity on the prevalence rate was partly accounted for by socioeconomic disadvantage; autistic children are 60% more likely to be from a socially disadvantaged household than those without an autism diagnosis. [8]. It is estimated that 700,000 people in the UK have a diagnosis of autism [9]

Autistic individuals may face significant challenges in day-to-day functioning which can have a notable impact on the individual themselves and their family [10]. The World Health Organisation has made autism a global health priority [11]. Autism and learning disabilities were also recognised as a priority in the NHS Long Term Plan in 2019 [12]

Most autistic children continue to face challenges into adulthood. Some autistic adults achieve independent living but often need significant family support with the challenges of adult life [13].

Higher levels of parenting stress are found in parents of young autistic children than parents of children with disabilities [14]. Parents can struggle with the daily challenges

of their child's autism characteristics which are often combined with additional conditions (e.g. Attention Deficit Hyperactivity Disorder), and physical symptoms (e.g. sleep disturbance)[14].

1.2.1 The aetiology of autism spectrum disorders

Understanding of the aetiology of autism is growing but one unifying cause or pathway is yet to be identified. The conclusions of the MRC Review of Autism research – Epidemiology and Causes in December 2001 were;

- It is widely thought that autism has a variety of causes across all those with a diagnosis

- Genetic research into autism suggests that genetic differences may increase autism likelihood

- It is not possible to exclude gene-environment interactions contributing to autism pathogenesis

- In a small percentage of cases, a probable medical cause can be identified and this is usually various genetic disorders (e.g. Rett's syndrome) or chromosomal abnormalities

Since the MRC review, a number of factors have been associated with the onset and progression of autism that appear to involve epigenetic changes [15]:

- Older age at conception of either parent

- Exposure to air traffic pollution by either the mother during pregnancy or the child during infancy

- Metals exposure during pregnancy for some metals

- Exposure during pregnancy to a number of pesticides

- Immune activation in the mother during pregnancy

- Obesity or gestational diabetes mellitus in the mother

- Elevated steroidogenic activities in the mother

- Maternal use of thalidomide, misoprostol and valproic acid and possibly selective serotonin reuptake inhibitors (SSRIs) either prenatally or neonatally

Adequate intake of folate has been identified as a protective factor, particularly if taken around 4 weeks before conception and in the first trimester [16]. There is some evidence to support the notion that good folate levels protect against the damaging effect of certain environmental pollutants on the developing brain [17]. The gut microbiota are known to be involved in detoxification of certain toxic substances and certain toxic substances are also known to impact on the gut microbiome [18].

1.2.1.1 Genetics and epigenetics

The heritability for autism has been estimated as 50 per cent [19]. There are many genes, chromosomal abnormalities, epigenetic changes, copy number variants (CNV), and de novo mutations [20] that correlate with an increased likelihood of autism [15]. There is a general acceptance of the existence of a genetic vulnerability to autism. ASD is a behavioural and psychological diagnosis, and those diagnosed form a clinically heterogeneous group such that the overlap between phenotypes has complicated the study of genetic influences. Genetic studies are beginning to converge on a number of genes that are highly involved in foetal brain development [21]. Despite the results of early twins research into the genetics of autism, currently only 10-20% of cases can be attributed to known genetic variations that are associated with autism [20]. This means that for most autistic people the cause of their autism cannot be attributed to a single genetic anomaly.

Epigenetics is the turning on and off of genes via methylation or histone changes, in response to environmental factors, and this has been considered as a possible mechanistic route for the effect of environmental factors on autism aetiology [22]. Epigenetic effects can happen in-utero and when this happens with twins, this may have led to an overstatement of heritability estimates for ASD [17]. The daily interaction between humans and their gut microbiome is a major exposure to the environment which is capable of triggering epigenetic changes [23].

To try and unravel the epigenetic effect, research has studied groups of genes involved in common pathways (e.g. synaptic plasticity) and compared gene

expression in autistic (AUT) groups to non-autistic (Non-A) groups. Using transcriptome analysis, Gupta et al [24] found two genes that were expressed differently in AUT samples compared to Non-A samples. The results of Weighted Gene Correlation Network Analysis support an interplay between the innate immune system and the nervous system in ASD aetiology: Interestingly, these are two body systems that the gut microbiome is tightly interconnected with.

1.2.1.2 Brain Structure

In a review of neuroanatomy in autism [25], the authors summarise a number of differences identified in autistic brains. One of the more consistent findings is a larger pre-frontal cortex in the early years. Other regions of the brain have also been found to differ in size and alterations have been found at the cellular level. It is unclear whether these differences contribute to the aetiology of autism or are a result of it.

In a large case-controlled study [26] autistic children had an average level of extra-axial cerebrospinal fluid 15.1% higher between the ages of 6 months and 3 years, than non-autistic controls, and this included both children that were at high and at normal genetic susceptibility of autism. They also had a larger average brain volume and these two factors independently correlated to a larger average head circumference. However, it is not clear whether these findings affect the development of the brain.

Autistic children have altered blood levels of brain-derived neurotrophic factor (BDNF) compared to non-autistic children but studies have found contradictory results, with some finding higher levels [27] and some finding lower levels [28]. BDNF is involved in synaptic plasticity and regulation of neural growth but the role of BDNF in the development of autism is not yet understood [27].

The cerebellum has been found to be structurally and functionally different in autistic individuals [29]. There are several gene mutations associated with increased susceptibility to autism on genes that are involved in the development of the cerebellum. The cerebellum of autistic individuals has been found to have active inflammation along with other areas of the brain (cerebral cortex and white matter) to a lesser extent [30].

Research has not yet defined a clear "autism" brain structure or neurology and it may not be found given the wide heterogeneity within the ASD diagnosis [25]. Given the

lack of this finding, it is still unclear whether brain anatomy and neurobiology contribute to the aetiology of autism or are a consequence of another aetiological pathway. Either way, it is yet to be proven.

1.2.1.3 Neuro-immune factors

Several alterations of the neuro-immune axis have been found in autism and these are discussed in detail in section 1.4.8 Immune Dysfunction. One of the current theories for the aetiology of autism is early inflammatory processes following immune alterations prenatally.

There are notable dysfunctions in peripheral blood mononuclear cells (PBMCs) and monocytes in autistic children. These immune cells are engaged in a long-term pro-inflammatory state, which affects immune system response. The altered PBMCs in autism lead to increased production of pro-inflammatory cytokines and subsequently to disruption of the blood-brain barrier. Alterations in the permeability of the blood-brain barrier can directly affect neuronal connectivity and function, and neural plasticity, which some have theorised could trigger autistic traits [31].

A large case-control study looked at maternal mid-gestation blood samples and cord blood samples from offspring who went on to be diagnosed autistic or not [32]. They compared the levels of 60 growth factors and cytokines and found that mothers of autistic children have immune dysfunction at 17-21 weeks' gestation, with systemic inflammation and elevated levels of many pro-inflammatory molecules. Immune cells such as cytokines play a role in foetal development including brain development.

The gut microbiota are a major modulator of the developing immune system in children aged 1 – 3 years and this is also a critical time for brain development. The gut microbiome is known to communicate with neuroimmune cells during brain development [33] via the various mechanisms of the microbiota-gut-brain axis (described in section 1.5).

1.2.2 Heterogeneity

It is accepted that ASD is a heterogenous condition both in terms of the impact of core autistic characteristics and also whether or not associated conditions are also present, such as learning difficulty and Attention Deficit Hyperactivity Disorder [34]. Applying

the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) guidelines, autistic traits need to cause a significant impairment in daily life to warrant an ASD diagnosis. Even given this, there is a wide difference in the amount of daily support an autistic individual needs, from those who need 24-hour care to keep them safe and those who can function with minimal or no support on most days. There is also a wide variety in speech and language abilities with some autistic children having no delay in speech and language development and others remaining non- or minimally verbal into adulthood. To say a person is mildly or severely impacted by their autistic traits, ignores the fact that many autistic people are not evenly impacted in all the domains of autism; a person may be severely impacted in the domain of speech, language and communication but mildly impacted in the domain of repetitive behaviour [35]. The impact of autism on the individual can change according to the level of stress and sensory stimulation in a particular environment [36] potentially confusing assessment based on their presentation on a particular day.

Genetic, epigenetic, neuroanatomy and metabolomic research is indicating a level of biological diversity amongst those with an ASD diagnosis. This is supported by the evidence of heterogeneity of autism over the lifespan of some individuals, including evidence that autism is not always present in the very early years but presents as a developmental regression in some children [37][38]. It is not a life-long diagnosis for all individuals since some move out of the cut-off for ASD diagnosis as they get older [39].

There is increasing evidence of a different presentation of autism in women and girls compared to men and boys. Autistic females are more likely to use "masking" or "camouflaging" to hide their autistic characteristics, like repetitive behaviours and also show better vocal expression than autistic males [40].

Such is the combined heterogeneity in autism that some have questioned ASD as a useful single diagnosis in research. Some associated conditions that have high prevalence in the autistic population, like epilepsy, anxiety, depression and gastrointestinal disorders, have been suggested as possible targets for investigation of autism phenotypes [34]. In support of this, there is evidence that treating depression in autistic children may reduce the impact of core autistic traits [41]. Common associated conditions are discussed in detail in section 1.4.

Heterogeneity in autism has led some researchers to restrict their study population to those without a learning disability (LD), without epilepsy and to those with spoken language. This questions the generalisability of the results to the wider autism population. Conversely, it has been suggested that the wide heterogeneity and no clearly defined autism phenotypes, may be hampering the detection of treatment effects in clinical trials targeting co-occurring conditions such as irritability and aggressive behaviour [42]. The wide heterogeneity adds complexity in choosing outcome measures suitable to all research participants and sensitive to change across the spectrum of participants.

1.2.3 Early mortality

A large Swedish matched case cohort study [43] found significant premature mortality in autistic people due to a multitude of medical conditions. They calculated that autistic people have a 2.56-fold increase in the odds of premature mortality compared to the population matched on geographic district, age, and sex. Autistic individuals who also have an intellectual disability (ID) die on average 30 years earlier and those without an associated ID die on average 16 years earlier. Most premature deaths are attributed to either accidents or co-occurring medical conditions such as epilepsy and intellectual disability [43][44].

1.3 Standard treatment for Autistic Spectrum Disorders

In the National Institute for Health and Care Excellence (NICE) guidelines [45], treatments are separated between those addressing the core features of autism and those that treat common associated health conditions. The core features of autism are difficulties in social skills and communication, plus the presence of very limited interests and/or repetitive behaviours. Social-communication interventions are recommended for autistic children to address difficulties with social interaction and communication.

Looking at common associated conditions in autistic individuals, the recommended treatment for challenging behaviour is psychosocial interventions [45]. Apart from this, there are no special recommendations for autistic individuals for symptoms such as insomnia, depression, anxiety, allergy and constipation, so guidelines for treatment are the same as the general population.

NICE have published guidelines for the management of Autism in under-19's. These recommend a detailed assessment of the care needed by autistic children and young people, and the development of a management plan which co-ordinates support from local specialist multidisciplinary teams. The guidelines state that each autistic child or young person should have a nominated professional who co-ordinates healthcare according to their plan and supports the individual through the transition to adult services. The care manager may be co-ordinating a number of professionals (speech and language therapist, paediatrician, occupational therapist, social worker, members of the local education authority or mental health professionals). Where a child's behaviour risks harm to themselves or others, or is impacting their ability to attend school, the guidelines recommend that they are assessed for precipitating factors (environmental factors like changing school or a new mental or physical health condition). Regular reviews and updates to the care plan are recommended for successful management of ongoing issues such as constipation, depression, self-harming, sleep problems and epilepsy [46]

Despite these guidelines, a report from Queen Marys University in 2016 [47] found parents of autistic children reported significant difficulty in accessing NHS services for their children for various health conditions.

1.4 Common associated conditions

Associated health conditions are common and varied in autistic children [47] and amongst them are gastrointestinal symptoms, which often co-occur with other conditions of increased prevalence in autism, including epilepsy, anxiety, intellectual disability and sleep disorders. Symptoms of co-occurring disorders and side effects of medications can become interwoven into a complex medical picture.

1.4.1 Gastrointestinal Symptoms and autism

There is a higher prevalence of gastrointestinal (GI) symptoms in autistic children compared with non-autistic children (Non-A) [1] and this appears to be consistent across ethnicities [2]. A review of studies looking at GI symptoms in autistic children, found the median prevalence rate as 46.8%, range 4 – 97% [48]. There is no standard validated GI symptoms assessment specifically for autistic patients which may account for some of the variability in the prevalence estimates. GI distress in minimally

verbal individuals may not be recognised, and the method of collecting the data (via medical records, parent survey etc) is also likely to contribute to the disparity in estimates. In a systematic review and meta-analysis, autistic individuals were found to be more likely than the general population to suffer any inflammatory bowel disease (OR 1.91), Crohn's disease (OR 1.37) and ulcerative colitis (OR 1.7) [49]. Some autistic individuals with GI dysfunction are also known to have altered carbohydrate digestion [50].

Functional constipation was found to be the most common type of GI dysfunction in autistic children (85%) [51], indicating that most GI disorders suffered by autistic children are a Functional GI disorder (FGID). A functional gastrointestinal disorder is a chronic GI symptom or symptoms where structural disease of the gastrointestinal tract is absent. In the general paediatric population, functional GI disorders often present with numerous accompanying symptoms (sleep problems, anxiety, severe stress, long-term fatigue, headaches, nausea and feeling dizzy [52] : poor sleep and anxiety are common in autistic children and show correlations with the presence of GI symptoms (see 1.4.5 and 1.4.7)

There is evidence that GI symptoms may present differently in autistic children (detailed in section 1.4.1.2). Parents of autistic children report that their children have difficulty verbally expressing when they are experiencing GI discomfort and report that they rely on stool appearance and behaviour change like irritability, as signs of GI distress [53]. In a consensus report, Buie et al list a number of behaviours that may indicate abdominal pain in autistic individuals [54]. The ASD Gastrointestinal and Related Behaviours Inventory (ASD_GIRBI) is a recently developed autism specific measure for GI symptoms which includes mealtime and non-verbal behaviour but this is yet to be independently validated [55] or widely used.

Gastrointestinal problems persist in a large proportion of autistic adolescents. In a 2-year follow-up study of 56 autistic participants (mean age 11 years), GI and sleep problems were seen to persist in the majority of the children and to commonly present together. At the follow-up, 64.3% suffered both GI and sleep problems; 14.3% sleep only and 8.9% GI only. At least one GI symptom was still present in 84.4% of participants at the 2-year follow-up, and 91.5% still had sleep problems. Unfortunately,

no record was taken on whether treatment for GI symptoms or sleep problems had been received [56].

In another study of 225 autistic children (aged 2-17 years), 25.8% suffered chronic abdominal pain at the beginning of the study and 86.7% of those were still suffering with this a year later. At the 1-year follow-up 23.8% had new symptoms of chronic abdominal pain [57].

In summary, the prevalence of gastrointestinal problems is high in autistic children, and they present somewhat differently than in non-autistic (Non-A) children. Given the evidence in Non-A children, GI problems may co-occur with a number of other symptoms in a complex interplay [52].

1.4.1.1 Gastrointestinal symptoms and impact of autistic characteristics

There is evidence that autistic children with GI disorders are more impacted by autistic characteristics [2][58]. In a study of children diagnosed with Pervasive Development Disorder (PDD), behaviours associated with autism showed a correlation to the presence of GI symptoms [59]: They found higher scores for irritability, anxiety and social withdrawal in the children with PDD and GI problems (mostly constipation and diarrhoea) compared to those with PDD and no gastrointestinal problems. However, they did not find a relationship between the presence of gastrointestinal symptoms and the impact of autism traits. In contrast, another study of children with a wider ASD diagnosis (including autism, Asperger syndrome and Pervasive Development Disorder/Not Otherwise Specified) found a strong positive correlation between the severity of GI symptoms and the impact of autistic traits, indicating that children with more severe GI symptoms were more impacted by autistic traits and vice versa [58]. A causal pathway for GI symptoms in contributing to the impact of core autistic traits is supported by the results of a small open study that used a polyethylene glycol (PEG) laxative for autistic children with chronic constipation. They reported a clinically relevant reduction in psychologist-assessed Childhood Autism Rating Scale (CARS) after 6 months, which is a measure of autism impact [60]. The mechanism of this improvement is not clear but it is known that PEG can alter the gut microbiome [60]. However, the authors report they cannot rule out some of the reduction in impact of autism being due to a reduction in pain and discomfort [60]. Similarly, a small open study of Microbiota Transfer Treatment (MTT) for autistic children with GI symptoms

reported a 22% improvement in CARS score and 80% reduction in GI symptoms at the end of treatment [61] and the participants' CARS score continued to improve at a two year follow-up [62]. These two studies were open, so the assessors were not blinded to the treatment but both employed psychologists for the assessments. The evidence that GI symptoms may be contributing to the impact of autism characteristics is interesting but needs more research.

1.4.1.2 The relationship between GI symptoms and behaviour in children

Some behaviours relating to anxiety and depressive disorders are known to be more common in the general population who suffer from functional GI disorders. However, it seems that autistic children with GI symptoms may display a different pattern of behaviours associated with GI disorders.

In non-autistic (Non-A) children, studies have mostly focused on anxiety and depression in those with GI symptoms. One study also looked at behaviour in relation to GI disorders and, found that Non-A children with functional constipation have a four-fold increase in behavioural problems (temper, non-compliance, aggression) compared to those without constipation. The authors suggest that functional constipation and related behaviours are inter-related and share the same complex, multifactorial pathophysiology [63]. Chronic constipation can drive behaviour and vice versa [64]. Although temper, non-compliance and aggression are seen in autistic children, these are not part of the core autistic characteristics.

Several studies have shown GI problems and anxiety to be highly correlated in Non-A children [57]. 40% of Non-A children diagnosed with functional abdominal pain (FAP) had a functional GI disorder (FGID) in adolescence. They also had significantly increased odds of both a lifetime or current anxiety disorder (OR 4.9 and 3.57) and a lifetime depressive disorder (OR 2.62) compared to adolescents that did not have FAP in childhood. So there appears to be a long-term vulnerability to certain psychological conditions from childhood abdominal pain. Whether the gut microbiota play a role in this vulnerability is as yet unknown although an altered gut microbiome has been identified in adults with depression [65] and anxiety [66]. There is also some evidence that GI symptoms in Non-A children may impair social skills: Non-autistic children assessed on initially presenting at a gastroenterology clinic had worse social skills than age- and sex-matched Non-A children without GI symptoms [67].

Anxiety and depression are common co-occurring conditions in autistic people (as detailed in section 1.4.7) and difficulty with social communication is a core feature of autism. However, there is evidence that GI symptoms present slightly differently in autistic children. Analysis of medical records for 487 autistic children, showed the presence of a GI symptom was significantly associated with all of the following; oppositional or destructive behaviour, sleep disturbance, delayed achievement of motor milestones and unusual eating habits [68]. In a large study using parent reports of persistent GI symptoms in their autistic child, there was a significantly higher incidence of sensory over-responsivity and anxiety in those with GI symptoms compared to those without, and a positive correlation between the number of chronic GI symptoms and the reported levels of anxiety and sensory over-responsivity. Sensory over-responsivity can present as hypervigilance, avoidant behaviour and distress. Anxiety and sensory over-responsivity were highly correlated and each independently contributed to the prediction of all GI symptoms except diarrhoea [69].

In a study of 108 autistic children, those with functional constipation (FC) 40% scored significantly higher on assessments for self-injurious behaviour, ritualistic behaviour, the need for sameness, and compulsive behaviour compared to those without GI symptoms. Some children with FC were unmedicated for constipation or behaviour and for this sub-group, rigid-compulsive behaviour did not associate significantly with constipation. This suggests that there may be a complex relationship between GI symptoms, common medications and certain behaviour in autistic children [70].

Chakraborty et al attempted to understand some of the complex interplay between GI symptoms and related behaviour, and core autistic traits in young autistic children (2 – 7 years) with GI symptoms. As stated above, anxiety, aggression, temper and non-compliance have been found to be related to GI symptoms in non-autistic children: After accounting for aggression, irritability and specific fears, Chakraborty et al found that the severity of gastrointestinal symptoms in autistic children was significantly related to the impact of autistic traits overall, and positively correlated with the impact of stereotypic behaviour and repetitive behaviour [71]. This suggests that either GI symptoms may contribute to the impact of these autistic traits or vice versa and raises the possibility that treatment for GI symptoms may reduce the impact of some autistic traits. Overall, the evidence is increasingly indicating that treating GI symptoms in

autistic children should bring benefits in wellbeing beyond improvements in GI symptoms.

1.4.2 Epilepsy

There is a significantly elevated prevalence of epilepsy amongst autistic individuals: Epilepsy is found in 21.5% of autistic individuals who also have an intellectual disability (ID) and in 8% of those without an ID. This compares to a prevalence rate of 0.97% in the general population [72]. At present there is no evidence that autism can cause epilepsy but they may share common pathways of aetiology including a genetic predisposition and the effect of environmental factors [73]. Following this hypothesis, gut dysbiosis and alterations in diversity appear to be involved in the development of epilepsy [74]. The ketogenic diet is known to improve epilepsy in children [75] and it has been found to alter the gut microbiota with a resulting decrease in the levels of the pro-inflammatory cytokine IL-17 [76]. Animal research indicates that the gut microbiota mediate the anti-seizure effect of the ketogenic diet [77] The ketogenic diet has been studied with autistic children and there is a small amount of evidence that it improves sociability and behaviour in autistic children [78]. This research supports the theory of a microbiota-gut-brain link in a sub-group of autistic children with epilepsy.

1.4.3 Intellectual disability

A large study in Sweden estimated the prevalence of intellectual disability in autistic children and young people as between 22-55% in 2017 [79]. A more recent estimate for school age children in England was lower, 18.1% possibly reflecting the change in diagnostic criteria for ASD in recent years [8]. A connection has been shown between cognitive functioning in young children and the gut microbiota: A cohort study profiled the microbiome of 380 healthy children aged 45 months and found a significant proportion of the variance in levels of cognition correlated to differences in microbiota composition [80]. Although this indicates a possible causal pathway between the gut microbiome and brain function, other environmental factors could be at play.

1.4.4 Speech and language difficulties

It is estimated that 25-35% of autistic people are minimally verbal, meaning they cannot communicate their needs verbally, and this complicates the reporting and assessment of gastrointestinal pain and symptoms and access to relevant healthcare

[81]. Difficulties with communication can vary for an individual autistic person according to the situation they are in: Physical stress, sensory overload, anxiety or illness can lessen the ability of autistic individuals to communicate [82]. Even when an autistic person is able to use verbal communication there are a number of challenges to effective communication between autistic people and non-autistic people which need to be considered when making a clinical assessment and these are detailed in Haydon et al [82]. This may lead to under-reporting of GI distress in autistic individuals.

1.4.5 Sleep disorders

Autistic children suffer a variety of sleeping problems and the prevalence of sleep problems in autistic children has been estimated between 40% to 80% [83]. Evidence indicates that sleep problems are persistent in this group [56] and there appears to be a link with GI symptoms: Autistic children with GI symptoms (AUT-GI) have higher odds ratios for any sleep problem and also for multiple sleep problems, than autistic children without GI symptoms (AUT-NoGI) [84]. In addition, autistic children with reflux have worse sleep problems than autistic children without reflux [85]. Hua et al studied the microbiome in AUT-GI compared to AUT-NoGI and found a significant difference in the abundance of two butyrate producing bacteria and a significant negative correlation between the abundance of these two bacteria and the degree of sleep problems suffered. Studying gut bacteria metabolites in the stool samples, they found significantly lower melatonin and higher serotonin in the AUT-GI group compared to the AUT-NoGI group. There was also a negative correlation between the levels of melatonin and the degree of sleep problems and a positive correlation between the levels of serotonin and the degree of sleep problems [86]. McCue et al comment that treating GI disorders may be an effective intervention for reducing the incidence of sleep problems in some autistic children [87].

1.4.6 Sensory processing differences

Both an over-sensitivity (hypersensitivity) and an under-sensitivity (hyposensitivity) of the senses have been reported in autistic individuals. A study of 25,627 autistic children aged 4 – 6 years old found 74% had sensory issues documented [88] and atypical sensory response is now referred to in the DSM-V description of ASD [10].

Sensory disorders in autistic children appear early in cognitive development: A reduced response to both social stimuli and non-social stimuli has been found in autistic children assessed as having a developmental age of 6 months compared to matched developmentally-delayed (DD) children and matched non-autistic without developmental delay children [89]. This altered sensory experience affects all the senses including taste, touch, smell and hearing but the mechanisms are unclear [90]. However, this could theoretically contribute to the increased incidence of toileting resistance found in autistic children compared to DD children [91] and may precipitate GI problems such as constipation.

Mealtimes and eating is a sensory experience and sensory issues have been found to partially predict food selectivity in autistic children [92]. Esposito et al found that food selectivity in autistic children appears to be secondary to sensory issues and that GI symptoms were associated with food refusal and a diet of limited variety [93]. The implications of a limited variety diet on GI symptoms and the gut microbiota are discussed in 1.6.

1.4.7 Anxiety and depression

The prevalence of an anxiety disorder in autistic children has been estimated at 40% [94] and a lifetime prevalence of anxiety disorders in autistic adults is estimated at 42% [95]. There are low response rates reported to standard anxiety treatments like Cognitive Behaviour Therapy (CBT) [96]. Using robust statistical modelling to explore the relationship between internalising symptoms like anxiety, and GI symptoms in autistic children, Dovgan et al found the best fit model indicated a bi-directional relationship between the two symptoms. [97]. This indicates that anxiety can affect GI symptoms and vice versa in autistic children and resolution of either may require the treatment of both.

Autistic children with chronic abdominal pain have higher levels of anxiety and greater sensory over-responsivity (SOR) than autistic children without chronic abdominal pain [57]. The presence of chronic abdominal pain could be predicted from the SOR score but neither the SOR score or the anxiety score predicted improvement in abdominal pain a year later [57]. This supports the argument for dual treatment of both GI symptoms and anxiety.

In a cross-sectional study, autistic children with GI symptoms had significantly higher affective disorder scores (which includes depressive symptoms) than autistic children without GI symptoms, although causation could be either way [98]. However, one should consider that there is no validated and reliable measure for assessment of depressive symptoms in autistic individuals, especially those with limited communication ability [99]. Perhaps unsurprisingly, there is a large variation in estimates for the rate of depression in autistic children and adolescents ranging from 0-83% [100].

1.4.8 Immune dysfunction

The immune system and the central nervous system (CNS) work together to regulate various body systems both in infant development and beyond, and a role has been proposed for the involvement of the immune system in the development and course of autism in at least a subset of children [101]. There is an established role for the immune system in the development of the foetal brain and the gut microbiome is known to be a major modulator of the immune system (as discussed in section 1.5.2).

A number of markers of immune system abnormality have been identified in autistic children when compared to non-autistic children including;

- Elevated plasma levels of pro-inflammatory cytokines (tumour necrosis factor alpha, interleukin-6 and interleukin-8) [102]
- Elevated serum levels of the pro-inflammatory interleukin (IL) 17A which correlated with the impact of autistic traits [103]. IL-17A plays a role in several autoimmune conditions with a neuro-inflammatory component
- A negative correlation between the impact of autistic traits in autistic girls and plasma levels of IL-8, interleukin 1 beta, vascular endothelial growth factor and macrophage inflammatory protein 1-alpha [104]
- A negative correlation between the severity of autistic traits and plasma levels of platelet-derived growth factor [104]
- Increased microglia and astroglia activation accompanied by a general neuroinflammatory picture in autopsied brains of autistic people [105]
- A pro-inflammatory profile of cytokines in the cerebrospinal fluid of autistic individuals [105]

- The presence of auto-antibodies to brain proteins in the mothers of autistic children [106]
- There is an increased rate of food allergy in autistic children compared to non-autistic children, weighted prevalence 11.25% vs 4.25%[107].

In summary, there is a picture of a pro-inflammatory status with a tendency to autoimmune reactions in autistic individuals. However, whether this contributes to the aetiology of autism or is a consequence of other factors and to what extent this involves the gut microbiome, is yet to be definitively established.

1.5 Microbiota-gut-brain axis related to autism

It has been hypothesised that the gut microbiome influences the presentation and impact of autistic traits. A high prevalence of GI symptoms are found in autistic children and the presence or severity of these symptoms have been shown to relate to the impact of autistic traits. Distinctive patterns of gut microbes and their metabolites have also been identified in autistic children [108] [109] [58]. In a systematic review of GI symptoms in autistic children, Leader et al confirmed a high incidence rate of GI symptoms and found some evidence supporting a causal effect for a gut-immune-brain pathway in autistic children [110].

The human intestines are host to a very large number of microbes including yeasts, bacteria, and parasites. There is currently no definition of what constitutes a healthy human microbiota [111] except that a gut microbiota consisting of highly diverse species is generally considered beneficial [112] and low diversity of species is associated with a number of disease conditions including Irritable Bowel Syndrome [113]. For the most part, current knowledge cannot determine whether reduced diversity of the gut microbiota is caused by disease or vice versa. However, in the case of Clostridium difficile infection, disease clearly results from a loss of resilience and diversity in the gut microbiota. One of the protective mechanisms of a diverse microbiota is its resilience following a stressor such as antibiotics.

The gut microbiota perform many functions that are beneficial to humans [114];

- The development of the immune system and the metabolic system

- The development and maturation of the infant endocrine system

- The generation of neurotransmitters in the gut

- Inhibiting the proliferation of pathogens

- Contributing to the digestion of food

- Influencing the distribution and absorption of dietary fat

- Synthesis of certain nutrients (vitamin K, biotin)

- The generation of short chain fatty acids (SCFAs)

- Repair and maintenance of the intestinal barrier

- Aiding the clearance of some xenobiotics

- Promoting intestinal motility

The gut microbiome is the combination of the gut microbiota and its genes, which plays a major role in immune system development and regulation. It also contributes to communication between the gut and brain and has become known as the microbiota-gut-brain axis (MGBA). The communication between the brain and the gut is 2-way: Via the autonomic nervous system (ANS) and the hypothalamic-pituitary-axis (HPA), the brain can influence gut function [111]. It has been shown that the brain-gut pathway is bi-directional in its influence: In a 12-year follow-up study, higher levels of anxiety in adults was a significant predictor of new onset functional gastrointestinal disorder (FGID) at the 12-year follow-up. Additionally, the participants with a FGID at the beginning of the study, had significantly greater levels of depression and anxiety at follow-up. The authors state that effective treatment of FGIDs may reduce the risk of developing anxiety or depression in the future [115].

Changes in the ecology of the gut microbiome and the immune system status in the GI tract affects brain function and higher-order behaviour via the gut-brain axis. The mechanisms of action involve direct connection via the vagus nerve of the intestinal epithelium to the CNS, and indirect connections via the immune system, metabolites generated by the microbiota and via enteroendocrine cells [116]. The gut microbiota

could modulate the gut-brain axis through a number of pathways ; endocrine, immune, neural, or epigenetic to name a few [117].

There is now robust evidence that the microbiota-gut-brain axis is bi-directional which offers the potential that gut microbiota could be modulated to influence behaviour and mental health.   Preclinical research has shown that the microbiome has a significant impact on fundamental patterns of behaviour and cognitive function, including stress response and sociability [118].   An altered pattern of gut microbiota has been reported in a number of diagnosed conditions including autism, schizophrenia and depression [119] [120] [121].

1.5.1 Early development

The colonisation of the human infant gut begins during the birth process. This early gut microbiome influences neural development in the infant through a number of pathways [117]. The gut microbiome changes significantly in the first three years influenced by a number of endogenous and exogenous factors including the type of birth, the type of infant milk (breastfed or bottle-fed), immune system status and diet once weaned. During this process some microbes are accepted by the immune system as having a "permanent resident status", known as symbionts [114].

Past the age of 3 years, the gut microbiome stabilises and will usually be subject to only subtle changes even over a number of years. There are some events that are known to cause a shift in the adult microbiome including a significant change in diet or taking prebiotic or probiotic supplements [121] [122].

Research on the effect of the gut microbiome on central nervous system (CNS) development relies on animal research. This indicates that the gut microbiome influences some aspects of development of the CNS, particularly in the hippocampus [117]. The development of neurons is known to be affected by environmental factors but the details of the involvement of the gut microbiome on the CNS development in humans is still for the large part unclear [117].

1.5.2 Immune pathways

Research evidence is conclusive for the gut microbiota playing an important role in initiating and shaping the immune system in humans [111]. The gut microbiome is

capable of affecting the levels of cytokines circulating in the body and this is known to have notable effects on brain activity [117].

Preclinical research shows that the gut microbiome has a strong influence on the maturation and function of microglia [123]. Microglia are the tissue macrophages of the CNS, and they respond to any pathological changes in the brain. In the process, they produce various free radicals and cytokines that can trigger neuro-inflammation. There is evidence of microglial activation in Alzheimer's disease, schizophrenia, [124] and autism [123].

Microglia play a key role in synaptic pruning that remodels the brain during infancy in response to stimuli and this process continues into adulthood [123]. Impairments in synaptic pruning disrupt the excitatory versus inhibitory balance of synapses, which may contribute to the development of neurodevelopmental disorders [123]. Germ-free mice have abnormal microglia and research using these has found that microglia are also significantly affected by a gut microbiome lacking ecological complexity. Even during adulthood, the microglia need the presence of a complex gut microbiome to maintain mature status and without this will revert to an immature status and defective function. Recolonization of germ-free mice with a diverse gut microbiota can partly restore the normal features of microglia. This research determined that short chain fatty acids (SCFAs) produced by the gut microbiota are regulating microglia homeostasis [124] and although it is animal research, it demonstrates that gut microbiota play a role in modulating the systemic immune system via the microbiota-gut-brain.

1.5.3   Gut microbial metabolites and the brain

Gut microbiota produce a wide range of metabolites some of which can reach the brain [111] including SCFAs. There are number of SCFAs produced by microbial fermentation of dietary fibre including acetate, butyrate and propionate. SCFAs have been found to play a role in a number of processes including endogenous serotonin production and protection of brain integrity, and their presence in human cerebrospinal fluid (CSF) opens the possibility that they may pass through the blood-brain-barrier (BBB) [111].

There is evidence that gut metabolites can induce changes in mood and deterioration in cognition via neuroinflammation. Gram negative bacteria release lipopolysaccharides (LPS) which activate microglia to produce pro-inflammatory cytokines leading to neuroinflammation. This is associated with depression, anxiety and reduced cognition [111] and is also negatively correlated to sociability in autistic adults (as discussed in 1.7).

1.5.4 Significant influences on the gut microbiome

Diet has been shown to be the most influential factor on our gut microbiota and substantial changes in the balance of macronutrients can rapidly alter the microbiota [125]. Changing to a predominantly plant-based or predominantly animal-based diet can reproducibly change the gut microbiota, their metabolites and their gene expression (microbiome) in adults [126]. Ketogenic diets are an example where the change in diet changes glutamate and GABA levels which can lead to a reduction in seizure frequency [111].

The quantity and variety of plant foods in the diet can affect the density and diversity of microbiota in the gut due to the presence of non-digestible plant components (fibre and polyphenols) which provide an energy source for gut microbiota [127]. However, response to dietary changes varies from person-to-person depending on their baseline gut microbiome [128]. As described in section 1.6, some autistic children eat a very restricted, repetitive diet which would be expected to impact the diversity of their gut microbiota.

In a longitudinal study in healthy adults, a 6-week high-fermented-food diet increased the alpha diversity of the stool microbiota and most of the new species were not identified in the fermented foods eaten. Fermented foods included unpasteurised yoghurt, unpasteurised sauerkraut, Kimchi, kefir and unpasteurised kombucha. There was a change in blood markers for the immune system indicating a reduction in inflammation and inflammatory response. The authors postulate that the new species were either picked up from the participants environment or were in the participant's gut at undetectable levels at baseline and flourished with the introduction of the high fermented food diet. In comparison, the parallel group following a 6-week high-fibre diet had no increase in alpha diversity and a mixed response in immune markers which

clustered around their baseline levels of inflammatory markers: those with raised levels of inflammatory markers at baseline showed an increase after the 6 weeks, with the rest showing a reduction in immune activation. The authors comment that their study shows that the gut microbiota can be modified by a diet intervention [129].

Antibiotics significantly alter the gut microbiota composition and have been associated with reduced cognition in animal research [111]. In a systematic review and 2-way meta-analysis, autistic children were found to have significantly more early exposure to antibiotics both pre- and post- natal and this is associated with a significantly increased likelihood of autism [130].

Probiotics appear to have great promise for influencing the gut microbiota and improving health [111] but there are many questions unanswered about dose, strains and duration. However, results in human clinical trials of probiotics have been disappointing compared to animal studies. In animal studies, genetic variability is controlled, and diet is tightly controlled so this may contribute to the very different outcomes of probiotic studies in animals compared to humans. Probiotics are explored more in section 1.8.

Research has found that people with more social connections have a more diverse gut microbiota, whereas anxiousness is correlated with reduced stool microbial diversity [131]. This may impact autistic individuals who find social situations challenging and anxiety is also common in this group (section 1.4.7). Genetics appear to have a limited effect on the gut microbiome: a study of Chinese adults found only significant heritability for two taxa, Desulfovibrionaceae and Odoribacter [132]. In a stool metaproteomic analysis of autistic children, their families and non-autistic controls, significant similarities were found between household members even when not genetically related. The reverse was also true; no significant similarities were found between genetically-related people would did not share a household [133].

1.5.5 Findings of Autism microbiome studies

There have been many studies of the gut microbiome and metabolome in autistic children [108][109][134] which have identified several imbalances compared to non-autistic children. The findings have been inconsistent regarding specific species abundance which is possibly due to different methodologies used for collection and

storage of samples and analysis techniques. However, it could also be a reflection of the heterogeneity within the autistic spectrum combined with the influence of different diets in different cultures. Despite this, it is generally accepted that there is a significant difference in the gut microbiome of autistic children when compared to a control group that is non-autistic and not siblings of autistic participants [135]. Preclinical studies suggest that the altered gut microbiome in autism can contribute to alterations in intestinal permeability and alterations in the enteric nervous system [136], and the emergence of behaviour associated with autism [137].

A study using 16s rRNA analysis of the bacterial and fungal microbiota found in the stool samples of 40 autistic children, showed "an altered microbial community structure" when compared to 40 non-autistic (Non-A) controls. At the genus level, they identified several differences in the relative levels of bacterial abundance, and also a high abundance of the fungal genus Candida compared to the Non-A control group. Unlike many other studies, they recorded whether the study participants suffered constipation or not and determined that constipation was not an influencing factor for the differences found between the gut microbiota of the two groups [138]. This is confirmed in a study of Chinese children where they compared stool microbiota of autistic children with constipation (C-AUT), autistic children without constipation (NC-AUT) and age- and gender- matched non-autistic children (Non-A). They found a similar pattern of microbiota differences between the Non-A group and the NC-AUT group as they did between all the autistic children (AUT) and the Non-A group, and identified a group of 24 genera whose relative abundance was highly predictive of autistic status. They identified a number of differences in relative abundance of genera between the C-AUT group and the NC-AUT group which may relate to the presence of constipation in autistic children and could cloud the picture in autism microbiome studies that don't account for the presence of gastrointestinal symptoms [135].

Rose et al [139] studied four groups of children; autistic with GI symptoms (AUT-GI), autistic without GI symptoms (AUT-NoGI), a non-autistic control group with GI symptoms (Non-A-GI) and a non-autistic control group without GI symptoms (Non-A-NoGI). They found the AUT-GI group had significantly higher scores for behaviours associated with autism compared to the AUT-NoGI group. They found significant

differences in the stool microbiome between the Non-A groups and the AUT groups regardless of whether they had GI symptoms or not. They also found significant differences in the microbiota at the family level between AUT-GI group and Non-A-GI group. Analysis of peripheral blood mononuclear cell (PBMC) cytokine production after several immune stimulation assays, showed an abnormal immune response in only the AUT-GI group when compared to the Non-A-NoGI group and also when compared to AUT-NoGI group. They summarised that their results suggest a relationship between the gut microbiome, the immune system and behaviour in autistic children with gastrointestinal symptoms [139].

There is evidence to suggest an unusual development of the gut microbiota in autistic children (AUT) compared to non-autistic children (Non-A): In Non-A children aged from 2 to 11 years, the alpha diversity of species in stool microbiota was found to increase with age, whereas there was no change in alpha diversity with age, in an age-matched AUT group [135]. A diverse microbiome promotes resilience following disruption e.g. after antibiotics, and is essential for immune system homeostasis [140].

More recent gut microbiome studies have found that different microbiota can perform the same function in different people, highlighting the importance of analysing the functionality of the gut microbiome rather than just the relative abundance of species. A metaproteomic analysis of stool microbiota in autistic (AUT) children compared to non-autistic children (Non-A) found increased levels of some metaproteins associated with clostridia bacteria and carbohydrate metabolism. Using network analysis of the proteomic data, they found notable differences between children more impacted by autistic traits and those less impacted by autistic traits suggesting distinctly different gut microbiome activity between these two groups. They found another notable difference between autistic children with, and without, GI symptoms and reported that the difference in microbiome activity between the Non-A group and the autistic group, could mostly be ascribed to those in the autistic group with GI symptoms [133]. These findings don't entirely align with the studies analysing species abundance but they do all find a distinct difference between AUT and Non-A gut microbiome.

A two-centre study in the USA (Arizona and Colorado) has found a site-specific effect on the gut microbiome in both AUT and Non-A children and this has a stronger effect than a measure of GI symptoms [141]. The strong effect of study site may be

contributing to inconsistent findings on the gut microbiome and autism. In their longitudinal analysis of a small number of AUT (n=7) and Non-A (n=9) children from Colorado, they found that the impact of autism-associated behaviour correlated with measures of gut microbiome diversity in AUT children. However they found no relationship between autism-associated behaviour and either diet or GI symptoms, or between the gut microbiome and diet and GI symptoms in AUT children [141]. Although the longitudinal study is small, it adds to the evidence for the gut microbiome composition having an effect on autism-associated behaviour and supports the theory that manipulating the gut microbiome may bring benefits in wellbeing beyond the GI tract.

1.6 Self-restricted diets and effect on GI symptoms and the microbiome

Autistic children have been shown to have several significant differences in fasting blood nutrient levels compared to age-matched non-autistic children, although the mean level of the nutrients in autistic children was generally within the published reference ranges [142]. A study comparing nutrient intake of autistic children to non-autistic children found no significant difference in the dietary fibre intake [143].

Food selectivity including food neophobia is more prevalent in autistic children than non-autistic children [144][145] and gives rise to concerns about nutrient sufficiency, GI symptoms and density and diversity of the gut microbiota. In a large study, the prevalence of avoidant/restrictive food intake disorder (ARFID) in autistic individuals was estimated as 21% [146]. Unravelling the possible effect of autistic traits on dietary choices, and the interplay between diet, gut microbiota and GI symptoms is yet to be fully determined. A large stool metagenomics study using data from two Australian datasets (99 autistic children – AUT, 51 siblings without an autism diagnosis – SIB, and 97 unrelated children not assessed for autism and with no diagnosis on record – UNR) found the AUT group had a less diverse and poorer quality diet than the SIB or UNR group, but the variability in the diet diversity was significantly wider in the AUT group [147]. They did not have data on complete GI symptoms but had single-timepoint stool consistency data, and they found an inverse relationship in the AUT group between diet diversity and stool consistency. They also found a positive relationship between the stool taxonomic diversity and diet diversity, but it was not possible to determine the direction of causation as both factors were predictors of each

other. The authors point out that a reduced microbiome diversity may promote behaviour effects in autistic individuals or conversely, autistic traits may restrict diet diversity and thereby affect stool microbiome diversity [147].

One study found that the presence of GI symptoms in autistic children was not associated with distinct dietary habits or medication status [148]. A recent study looked at the associations between nutrient intake and GI symptoms in 120 autistic children: They found no associations between the intake of macronutrients, micronutrients or omega-3 fats and GI symptoms in autistic children [149].

1.7 Increased intestinal permeability and autism

Increased intestinal permeability has been shown in 25.6% of autistic (AUT) children compared to 2.3% in non-autistic controls [150]. Earlier research indicated that increased intestinal permeability in AUT children does not correlate to faecal calprotectin or GI symptoms [151][152]. However a positive correlation has been found between autism impact and levels of stool calprotectin [153] suggesting a possible link between inflammation in the gut and autism impact. Intestinal permeability testing in autistic children indicates some damage to the tight junctions [151]. Occludin is a protein that stabilises tight junctions and modulates intestinal permeability. Serum occludin levels are significantly lower in autistic children compared to non-autistic children and the serum occludin level in autistic children negatively correlates with the mean CARS score suggesting a link between poor intestinal barrier function and the impact of autistic traits [154].

In their systematic review of risk factors for increased intestinal permeability, Leech et al found associations with high fat or high protein diets, obesity, diabetes, liver disease and systemic inflammation. They concluded that the presence of increased intestinal permeability suggests a chronic disease state rather than a gastrointestinal condition [155]. Increased intestinal permeability can facilitate the translocation into the bloodstream, of gut microbes or associated metabolites such as lipopolysaccharides (LPS). Increased levels of serum LPS have been found in autistic adults, compared to age- and gender-matched non-autistic adults and this is inversely correlated to sociability scores [156]. Bacterial metabolites such as LPS that present in the bloodstream generate an immune response and research has shown systemic

inflammation in autistic individuals [157] (as discussed in section 1.4.8). Research suggests that a gluten- and dairy-free diet may have a protective effect against increased intestinal permeability in autistic children even when coeliac disease has been ruled out [152].

1.8 Probiotics and their effects relevant to autism

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [158]. Most probiotics are bacterial in nature but the yeast saccharomyces boulardii is also considered a probiotic. Live fermented foods and drinks like unpasteurised cheese, kombucha, sauerkraut, kimchi, kefir and yogurt are also probiotic in nature.

In recent years interest has flourished in the potential for probiotics to be used for the treatment of disease or as a protective factor. This has been fuelled by the increasing evidence for the multi-faceted role of the gut microbiome in health and disease. The mechanisms of action of probiotics have been reviewed [159][160] and most of the evidence comes from pre-clinical research. Different species, and even strains within a species, have different mechanisms and actions, and the action can be elicited in response to the microbe itself and also in response to metabolites produced by live microbes e.g. butyrate [160]

A major effect of probiotics is to modulate the immune and inflammatory systems in the gut and beyond. This can be by direct interaction with the immune system in the gut, or by altering the ecology of the gut microbiota, and thus it's metabolites, with subsequent alterations in interactions between the immune system and the gut microbiota [160]. Often this is by down-regulating an over-stimulated inflammatory response and clinically this has been shown to reduce allergic response and reduce symptoms of a number of inflammatory diseases including celiac disease, pouchitis, Parkinson's disease and Irritable Bowel Syndrome (IBS) [160]. Certain probiotics have been shown to improve the integrity of the tight junctions between epithelial cells in the gut which aids maintenance of normal intestinal permeability. This can reduce the damage effected on the intestinal barrier by some pathogenic gut microbes and their metabolites [157]. This may be one of the mechanisms for the anti-inflammatory effect of probiotics.

Certain probiotics have been shown to alter the enzymatic activities of the gut microbiota. An example of this is their influence on the nitrogen metabolism of gut bacteria that results in the production of the toxin p-cresol [121].

Of particular relevance to autistic individuals is the interaction between probiotics and the central nervous system (CNS), the mechanisms of which are not well understood. Some gut bacteria produce neurotransmitters or precursors to neurotransmitters including tryptophan, dopamine, noradrenaline and gamma-aminobutyric acid (GABA) [161][140] but their exact effect on the brain is unclear [159]. However the immune system and enteric nervous system are intricately linked to the central nervous system (as described in section 1.4.8) and the immune- and microbiota- modulating effects of probiotics have an impact on the brain via the various mechanisms of the microbiota-gut-brain axis [160].

1.8.1 Animal autism microbiome studies

In rat research [162], intracerebroventricular delivery of the SCFA propionic acid (PPA) induced a range of changes, closely resembling behaviour, brain imaging and neuroinflammatory markers (e.g. increased oxidative stress, reactive astrocytes and activated microglia) found in autistic individuals. All these changes were reversible. Further work by MacFabe identified a downstream effect of mitochondria dysfunction in the PPA-treated rats [163]. SCFAs are considered part of the microbiota-gut-brain axis and are known to reduce gut motility, modulate the immune system and affect mitochondria function [139].

Altered faecal levels of short chain fatty acids have been identified in autistic individuals [157] [164] but there has been some inconsistency about the nature of the alteration amongst studies. SCFAs are produced in the gut by the certain microbiota following fermentation of indigestible carbohydrate. Lui et al found the levels of faecal SCFAs in Chinese children correlated closely with the levels of SCFA-producing bacteria and the intake levels of indigestible carbohydrates [164]. They did not find altered levels of PPA in autistic children compared to non-autistic and postulate that inconsistent findings in other studies may relate to variations in gut microbiota in different study participant groups and methodological differences [164].

In animal models there is evidence that probiotics can have an effect in the brain. VSL#3 probiotic was administered to rats and subsequently their brain tissue was analysed for changes. The analysis identified an alteration in the expression of several genes involved in metabolic pathways that modulate inflammation and affect neuronal plasticity [165]. Although preclinical research, it raises the possibility that a probiotic could alter the gut microbiota and positively impact on neuronal health.

Preclinical research has shown that the anti-inflammatory effect of probiotics reaches the brain. In a rodent model of systemic inflammation, it was found that VSL#3 probiotic can reduce two inflammatory markers in the brain (microglial activation and monocyte infiltration) and also reduce systemic inflammation as shown by reduced levels of tumour necrosis factor alpha (TNF-*a)* [166]. The results suggest that if there is inflammation present, probiotics may benefit brain health. However, this is rodent research using a particular model of systemic inflammation and this may not translate widely to humans.

In the maternal-immune-activation (MIA) mouse model of autism, the administration of Bacteriodetes fragilis (B.fragilis) bacteria reduced the social deficit of the offspring, repaired intestinal hyper-permeability and altered the gut microbiota composition [167]. Maternal immune activation is a proven factor that increases likelihood of a later diagnosis of autism in the child, and increased intestinal permeability along with altered gut microbiota is common in autistic children. However, promising results in autism animal models have so far had poor outcomes in human clinical trials and B.fragilis is yet to undergo any human clinical trials in autistic individuals.

In the Shank3-knock-out (Shank3 KO) genetic mouse model of autism there is reduced alpha diversity of stool microbiota compared to wild-type mice, and a reduced expression of GABA receptors in the hippocampus, which positively correlated with stool levels of Lactobacillus reuteri (L.reuteri). The administration of L.reuteri increased expression of GABA receptors; improved social behaviours in the male mice; reduced repetitive behaviours in male and female mice; and reduced previously elevated interleukin-17a (a pro-inflammatory cytokine) in male mice [168]. The results indicate that where there is a genetic basis for autism-associated behaviours, it may be possible to modify these with a probiotic. There is currently a clinical trial being

conducted assessing the effectiveness of L.reuteri on sociability in autistic children [169].

In summary, the evidence of animal studies offers great promise, but animal models of autism are very specific and do not cover the heterogeneity seen in the human population with an ASD diagnosis. Whether this promise will be replicated in human studies is yet to confirmed. It may need the discovery and definition of biological sub-groups of autism or personalised probiotic intervention based on gut microbiome/metabolome results before we see similar benefits in human probiotic clinical trials.

1.9 Studies manipulating the gut microbiota in autism

Current evidence suggests that it may be possible to use probiotics to modulate the gut microbiota of autistic children but published studies manipulating the gut microbiome in autism are few and far between. In 2000, a small clinical study of vancomycin treatment in 11 regressive-onset autistic children reported some improvements in communication and autism-associated behaviour but these were lost when the treatment was stopped [170]. Since then, there have been six probiotic studies, two pilot studies, one case report of a probiotic improving social behaviour, one clinical trial of microbial transfer therapy and one clinical trial of a prebiotic with autistic children have been reported, which are summarised in Table 1-1.

Table 1-1; Probiotics, prebiotics and microbial transfer therapy studies in autistic children

Study authors	Year	No. in study	Study Type	Intervention details	Dose/duration	Reported results	Limitations
Santocchi et al [171]	2020	63 AUT	Probiotic, RDBPC clinical trial	Vivomixx	1st month 9×10^{11} CFU daily, then 4.5×10^{11} daily for 5 months	No significant difference in autism severity compared to placebo. Clinically and statistically significant reduction in ADOS CSS in non-GI subgroup compared to placebo. Improvement in GI symptoms, sensory processing and adaptive functioning in GI subgroup compared to placebo.	Large dropout rate, including 50% in the GI symptoms subgroup meaning only 17 completed from this subgroup
Sanctuary et al [172]	2019	8 AUT with GI symptoms	Probiotic + prebiotic, randomised double blind controlled crossover pilot	Bovine colostrum prebiotic (BCP) or Bifido infantis+ Bovine colostrum prebiotic	5 weeks each arm with 2 weeks washout between, BCP 0.15g/lb body weight, B.infantis 20×10^{9}	Treatment well tolerated. Improvements in GI symptoms in both treatment arms. Significant reduction in ABC score with BCP-only treatment.	Pilot study so small sample size
Arnold et al [173]	2019	10 AUT with GI and anxiety symptoms	Probiotic, randomised pilot crossover	VISBIOME	9×10^{11} to 1.8×10^{12} CFU, 8 weeks each arm, 3 weeks washout	All outcome measures improved after treatment. PedsQL score showed relationship with abundance of lactobacillus in stool. Significant improvement in GI symptoms on probiotic.	Pilot study so small sample size and no statistically significant results
Grimaldi et al [174]	2018	14 AUT + 16 AUT controls	Prebiotic, RDBPC trial, 4 groups	Bimuno ®GOS and exclusion diet (n=6) or Bimuno ®GOS	6 weeks, 1.8g 80% GOS content	Exclusion diet group; reduced abdominal pain and better bowel movements, correlations between faecal bacteria and faecal amino acids, improvements in anti-social	Small sample size of each study group.

				and unrestricted diet (n=8)		behaviour; increase in Lachnospiraceae	
Shaaban et al [175]	2017	30 AUT	Probiotic + prebiotic, open label clinical trial	Dried carrot powder + 2 strains of lactobacillus + Bifidobacteria longum	3 months, 5g powder daily = 500 x10^6 CFU	Overweight patients lost weight and reduced their BMI; increase in faecal colony counts of Bifidobacteria and Lactobacillus; ATEC total and subscores reduced; improvement in total 6-GSI score	Open label, no controls; patients received behaviour therapy alongside the treatment
Kang et al [176]	2017	18 AUT + 20 non-A	Microbiota transfer therapy clinical trial	2 weeks antibiotics, bowel cleanse, 8-week fecal transplant protocol	10 weeks + 8 week follow-up	Increase in stool bacteria diversity; increased abundance of Bifidobacterium, Prevotella and Desulfovibrio; 80% reduction in GI symptoms, autism-related behaviours improved	Open label study, small study
E.Grossi et al[177]	2016	1 AUT with ID	Probiotic, single case study	4 lactobacillus + 3 Bifidumbacteria + 1 streptococcus strain	4-month treatment, dose not published	Improvement in social affect score on ADOS-2	Single case study
Tomova et al [178]	2015	10 AUT + 9 Non-A siblings + 10 Non-A	Probiotic, cohort study	3 lactobacillus, 2 Bifidumbacteria, 1 streptococcus strains	4 months, 1 capsule 3x daily, Children Dophilus	Reduction in amount of fecal Firmicutes, Bifidobacterium and Desulfvobrio; Bacteriodetes : Firmicutes ratio normalised;	Small study size. Did not repeat CARS assessment after treatment so unknown effect on sociability and behaviour

West et al[179]	2013	33 AUT	Probiotic, caregiver survey	Delpro® probiotic + Del Immune V®	Recommended dose 21 days, 1×10^9 CFU 3x daily	Decrease in mean ATEC total score and all ATEC subscores	No control group. Dosing schedules administered by caregivers and compliance not reported.
Kaluzna-Czaplinska & S. Blaszczyk [180]	2012	22 AUT	Probiotic, cohort study	Lactobacillus acidophilus Rosell-11	2 months, 5×10^9 CFU twice daily	Better at following instructions and concentrating; differences in stool consistency	No description of how behaviour was assessed
Parracho et al [181]	2010	22 AUT	Probiotic, RDBPC crossover trial	Lactobacillus plantarum WCFS1	3 weeks, 4.5×10^{10} daily, 3 week washout	Increase in faecal Lactobacilli and enterococci bacteria counts; reduction in clostridia cluster XIVa bacteria counts; differences in stool consistency, improvement in antisocial behaviour, anxiety and communication problems vs baseline	High dropout rate, did not target subgroup within the autism diagnosis.

Abbreviations: AUT autistic children; Non-A non-autistic children; ABC Aberrant Behaviour Checklist; RDBPC randomised double-blind placebo-controlled; CFU colony forming units; CARS Childhood Autism Rating Scale, GOS galactooligosaccharide, ID intellectual disability; B. infantis Bifido infantis

Note: Delpro ® contains Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbruecki, Bifidobacteri longum, Bifidobacteria bifidum. Del-Immune V® is a lysed, lyophilized powder that contains peptidoglycan, muramyl peptides and nucleotide-containing components derived from Lactobacillus rhamnosus V strain. Bimuno is a brand name.

The small open-label trial of microbial transfer therapy (MTT) showed an 80% reduction in GI symptoms for autistic children and a significant improvement in autism-related behaviour after treatment [176] and both remained improved 2-year after treatment, with the notable differences in the gut microbiota after treatment, also maintained [182]. Analysis of participant stool samples showed an increase in the diversity of bacterial species post treatment and a significant increase in relative amounts of three particular species (Desulfovibrio, Bifidobacterium and Prevotella). Most notably, the relative levels of Bifidobacterium showed a 4-fold increase putting them at comparable levels to non-autistic children who had not received MTT. However, as an open-label study some results may be subject to a placebo effect.

A detailed review of the five early probiotic studies with autistic children (excluding Sanocchi et al) was reported by Patusco and Ziegler [183]. Four out of the five probiotic studies reported improvements beyond GI symptoms but they all have methodological issues (small sample size, high drop-out rate, non-standard measures, no control group). Three out of the five probiotic studies used a multi-species probiotic and two used a single strain probiotic. Parracho et al [184] used a double-blind, placebo-controlled crossover trial but the other four are non-controlled studies. Three of the probiotic studies included an assessment of behaviour before and after treatment, two used the Autism Treatment Evaluation Checklist (ATEC) and one used a different measure. All three noted a reduction in the impact of autism characteristics after probiotic treatment, although they did not control for concurrent therapies. Three studies assessed gut microbiota pre- and post- intervention via stool analysis and one via urine metabolites. All showed a change following probiotic treatment. A 2018 randomized, double-blind placebo-controlled trial of prebiotics, found that those in the prebiotics + exclusion diet group had improvements in anti-social behaviour along with a change in stool microbiota and urine metabolites, following treatment [174].

Using polymerase chain reaction (PCR) stool analysis Tomova et al [185] reported several differences in the faecal microbiota of autistic children compared to controls (9 non-autistic siblings and 10 non-autistic children) Results included the Bacteriodetes/Firmicutes ratio was significantly lower. The Childhood Autism Rating

Scale (CARS) was used to measure the impact of autistic traits. The autistic children took a daily multi-species probiotic (3 lactobacillus strains, 2 Bifidum bacteria strains and 1 streptococcus strain) for 4 months. Results following treatment included a significant reduction in the abundance of Firmicutes, resulting in an increase in the Bacteriodetes/Firmicutes ratio, matching the ratio of the control group. This was a small study and there was no after-treatment CARS assessment so it is unknown whether the probiotic changed the CARS score which would indicate a wider improvement in global function.

An autistic boy with a learning disability had an improvement in social behaviour after taking a multi-species probiotic for 4 months [177]. During this time there were no changes to the child's diet or well-established education programme. The impact of autism traits was measured using the Autism Diagnostic Observation Schedule-2 (ADOS-2) at six time points; twice before taking the probiotic, twice during treatment and twice after completing treatment. Following treatment, the severity of his GI symptoms were reduced. There was also an unexpected 3-point reduction in the ADOS-2 Social Affect domain (from 20 to 17) and this improvement was maintained for 10 months. Although a single case report, the ADOS-2 is the gold standard measure for autism impact and other potentially confounding variables of diet and other therapies were controlled for.

One of the few controlled studies was conducted by Parracho et al [181]. This was a double-blind placebo-controlled crossover trial of Lactobacillus plantarum WCFS1. Compared to placebo, probiotic treatment showed;

- an identifiable change in the pattern of faecal bacteria
- more frequent formed stools
- reduction in anxiety, anti-social behaviour, withdrawn behaviour and communication issues
- no significant difference in social relating behaviour

This study suffered a very high dropout rate, meaning that the results may suffer a bias towards improvement if those not seeing an improvement dropped out.

It is interesting that another case control study [186] of a single strain probiotic (Lactobacillus acidophilus strain Rosell-11), did not find a change in social relating behaviour (eye contact, response others emotions), but reported improvements in concentration and following instructions. However, the assessments used for the outcomes are not stated so these may not be validated measures.

Since Patusco and Ziegler's systematic review, the results of one further autism probiotic study has been published [171]. In this study there was no significant difference between the placebo and probiotic for the mean change in Total ADOS calibrated score (ADOS-CSS). An exploratory subgroup analysis showed a clinically significant reduction in Total ADOS-CSS in the no-gastrointestinal symptoms (NGI) subgroup following probiotic treatment and this was statistically significant compared to placebo. In the gastrointestinal symptoms (GI) subgroup they found a statistically significant improvement in GI symptoms and adaptive functioning compared to placebo. However, there was a high dropout rate in the GI subgroup such that only 17 out of 30 completed the study so the findings in the subgroup may be biased. The authors comment that the different findings in the two subgroups suggest that the action of probiotics may differ in autistic children with and without GI symptoms [171]

In summary, it is too early to dismiss probiotics as not effective for autistic children, despite the fact that two out of the 3 double-blind studies did not find a significant difference in their primary outcome between placebo and probiotic. There are still only a small number of studies reported, and all but Santocchi et al are small studies. Autism research studies have also suffered from a lack of a validated outcome measure that will reliably reflect an improvement in global function and wellbeing across the spectrum with an ASD diagnosis.

1.10 Conclusion

Autism spectrum disorder is an observational diagnosis and there is still no biomedical test for this diagnosis. There is wide heterogeneity in those with an ASD diagnosis and many common co-occurring conditions including GI disorders. In the future, separate phenotypes within the ASD diagnosis may be defined (as has happened in

diagnostic conditions like irritable bowel syndrome and dementia) and the co-occurring conditions have been suggested as a starting place for investigating such phenotypes.

Given the increased prevalence of GI disorders in autistic individuals and the increasing evidence of a role for the gut microbiome in affecting mood and cognition, Table 1-2 hypothesizes on the possible mechanisms of change for dietary interventions for autistic children with GI symptoms.

Table 1-2; Possible mechanisms of change for dietary interventions for autistic children with GI symptoms

Factors affecting drivers	Genetic predisposition	Epigenetic changes	Immune dysfunction	Antibiotics, pre- and probiotics	Diet	Altered levels of neurotransmitters
Possible drivers	Anxiety	Sleep disorders	Toileting behaviours	Altered gut microbiome	Allergies	Poor carbohydrate digestion
Impacts of autism and gastrointestinal disorders	Difficulties with social communication	Repetitive behaviours	Speech or receptive language difficulties	Altered sensory responses	Gastrointestinal symptoms	Restricted interests

The mechanisms of change for personalised dietary intervention are likely to be multifactorial and may include correcting nutrient deficiencies, reducing allergic response, improving bowel function and possibly promoting a change in the ecology of gut microbiota. As discussed in section 1.4, there are several common co-occurring conditions in autistic children such as GI symptoms, sleeping problems, developmental regression, and allergies.   Increasingly research into factors influencing enteric and central nervous system development and function are revealing factors which are also implicated in the development of GI disorders in autistic individuals [187].

It is possible that correcting nutrient deficiencies has a direct effect on some of these co-occurring conditions, for example correcting iron deficiency has been reported to ease sleeping problems [188]. Supplementing with a probiotic may ease GI symptoms and altered the ecology of the gut microbiota as discussed in section 1.9. A change in the gut microbiome may reduce activation of the immune system (section 1.2) and subsequently reduce inflammation and this effect may reach the brain (section 1.5.3). Similarly, removing suspected allergens from the diet may have reduced immune response and inflammatory tendency.   Ensuring adequate hydration and increasing the fibre intake may reduce constipation and abdominal discomfort and may have further effects on behaviour [63]. Expanding the range of colourful vegetables and fruit in the diet towards a Mediterranean diet, increases the range of polyphenols consumed and should lead to an increase in gut microbiome diversity [189].

Chapter 2   Assessment of personalised dietary intervention in Autistic children - a prospective study

2.1.1 Introduction

Autistic children are vulnerable to gastrointestinal dysfunction, poor digestion of carbohydrates and increased intestinal permeability. This section presents data from my private clinical practice (as described in section 1.1) to investigate the effects of personalised dietary intervention on the overall health and function of autistic children using the Autism Treatment Evaluation Checklist (ATEC) as the primary outcome measure. My hypothesis was that analysis of the before and after outcome measures would indicate an improvement in global wellbeing and functioning after personalised dietary intervention. A further hypothesis was that the change in ATEC Total was not simply due to a change in GI symptoms as measured by the Buie assessment and the ATEC subscale Health Physical Behaviour. A post-hoc analysis would also be conducted to explore the characteristics of those research subjects that had a positive response to the intervention. A discussion of the validity and limitations of the ATEC is presented in section 2.1.6.1.

2.1.2 Materials and methods

This analysis was based on a repeated-measures design. Participants had all taken part in personalised nutritional therapy as part of routine clinical practice at a private Environmental Medicine clinic. As part of the terms of engagement at the clinic the parents consented for their child's anonymised data to be used for clinical audit and research purposes. The aim of the personalised dietary intervention used in the clinic was to improve the nutritional quality of the child's diet while removing potential irritants to the gut and known allergens. Parents of the participants were guided to alter the child's diet over time with the following aims:

- increase the quantity and range of fresh vegetables

- increase the range of fresh fruit (intake was usually sufficient)

- remove artificial colours, preservatives and flavourings from the child's diet

- ensure adequate water intake (appropriate to age and level of physical activity)

- minimise the intake of refined sugar and refined carbohydrates

- include health-promoting oils in the child's diet e.g. extra virgin olive oil

- most participants (n=37, 62%) were also recommended to avoid foods containing gluten, dairy and soya for a period as an assessment of tolerance

The dietary recommendations for patients were individualised to the symptoms, health history and personal taste preferences of the patient, and suggested recipes were provided. Indications for recommending a trial of a gluten-free diet were autoimmune disease in the family; a diet dominated by gluten-containing food coupled with hyperactivity; parents reporting the child being "spaced out" after eating bread or pasta; or bloating and discomfort following eating bread or pasta. Indications for recommending a trial of a dairy-free diet were eczema, persistent constipation or diarrhea, or family members with dairy allergy or lactose intolerance. Soya exclusion was recommended where a gluten and dairy-free diet was indicated by the criteria above, and the child also had a history of allergies or eczema.

Table 2-1; Dietary interventions

Dietary Intervention	Number of patients n=60	Percentage %
Gluten, casein & soya free diet	37	62
Gluten & casein free diet	8	13
Gluten free diet	6	10
Other[1]	6	10
Probiotics (all)[2]	42	70
Ecodophilus	27	45
Symprove	10	17
Other probiotic	9	15
Calcium and/or magnesium	22	37
Multivitamin	25	42

Fish oils	27	45
vitamin D	7	12
Medium Chain Triglyceride oil	6	10

[1]Other prescribed diets including specific sugar diet and specific exclusive diet

[2]Some participants were prescribed two probiotics

A probiotic supplement was often recommended (70%, n=42). Indications for recommending a probiotic were irregular bowel movements, altered stool consistency, excessively odorous stools and eczema. The choice of probiotic was based on clinical experience, price and what the child was most likely to take (taste and format).

Nutritional supplements (e.g. calcium, vitamin D, fish oils) were recommended according to need, after an assessment of current dietary intake (particularly vegetables, oily fish and sources of calcium), analysis of any test results and assessment of signs and symptoms of nutrient deficiency. Any child following a dairy-free diet was prescribed a calcium supplement. Over 90% of participants had at least one nutritional supplement. Summary details for diet and supplement interventions are given in Table 2-1. As part of standard clinical practice, the patients were monitored and typically followed up after 12 weeks. The information available in the clinic database included a parent-completed medical history questionnaire, parent-completed ATEC and Buie assessments, the clinician's consultation notes, treatment plans, parent-completed follow-up reports and some laboratory test results. The ATEC is a measure of global wellbeing and function in autistic children and a higher score denotes poorer wellbeing and more impact on global functioning. The Buie is an unvalidated clinical tool used to assess possible abdominal pain in autistic children based on Table 2 in "Evaluation, Diagnosis and Treatment of Gastrointestinal Disorders in Individuals with ASDs: A Consensus Report" [54]. Examples of the ATEC and Buie assessments are given in the Appendices.

Sixty patients were independently selected from the clinical database as research subjects The selection criteria was that the patient had an autism diagnosis together with a record in the clinical database of a before and after score for at least one of the chosen outcome measures. The outcome measures that had been chosen were the ATEC total and subsection scores, the Buie score, and two urine organic acid markers - 4-Hydroxyphenylacetic acid and 3-(3-hydroxyphenyl)-3-hydroxypropionic acid

(HPHPA), (details of 4-Hydroxyphenylacetic acid and HPHPA are given in section 2.1.2.1). Anonymised data on individual characteristics, symptoms, dietary intervention plan and outcome measures for these 60 patients was independently extracted from the clinical database and input into an Excel spreadsheet. This formed the dataset for this analysis.

The outcome measure values before (baseline) and after (follow-up) the dietary intervention were compared to explore the effectiveness of personalised dietary intervention. A post-hoc analysis was completed to explore whether any particular characteristic differentiated the response to dietary intervention. In this analysis, a reduction of greater than 7 points in ATEC total score was considered a positive response (n=18). This is based on clinical experience and the fact that in the normative interpretation guidelines for the ATEC Total, the centiles in the mid-range (20th to 69th) amount to a range of 7 or 8 ATEC Total points per centile. Therefore, a change of more than 7 points would move the child into the next centile. Those with a reduction in ATEC total score of 7 or less, or an increase in ATEC total score, were considered Non-response (n=11). A second post-hoc analysis of responder characteristics used the Buie score as a measure of response to intervention and a reduction in the Buie score of greater than 5 points was considered a positive response (n=14). This is based on clinical experience as this is an unvalidated clinical tool. A reduction of 5 points or less, or an increase in the Buie score is deemed as Non-response (n=15). The research subjects were divided into responders and non-responders based on these criteria, and comparisons made between the characteristics of the Responder and Non-responder groups.

2.1.2.1 Organic acid test

The organic acid test analyses urine metabolites which can be used for screening for metabolic disorders and can also provide helpful insight on symptoms displayed in autistic children with gastrointestinal symptoms. The organic acid test used in this study is provided by Great Plains Laboratory. Two of the markers from this test, 4-Hydroxyphenylacetic acid (4-HPAA) and HPHPA, were chosen as measures for assessment of the effectiveness of dietary intervention. Elevated levels of HPHPA is associated with relative over-abundance of Clostridia bacteria in the intestines and elevated levels have been found in autistic children compared to non-autistic children

(matched for age and gender) [190]. Elevated levels of 4-Hydroxyphenylacetic acid is found in small intestinal bacterial overgrowth (SIBO) [191]. Where these two metabolites are elevated at baseline compared to the reference values provided by Great Plains Laboratory, a reduction in level was considered an indicator of effectiveness of the dietary intervention.

2.1.3 Data and statistical analysis

Complete data for the ATEC total score was available for 29 research subjects. The data for these research subjects was analysed to assess effectiveness using first the ATEC as a measure (Total and subscale scores) and secondly the Buie score. 29 research subjects had complete Buie results that could be used in this analysis.

The null hypothesis for all outcome measures was that there is no difference between the mean at baseline and the mean at follow-up. For all null hypotheses tested, statistical significance was set at alpha = 0.05. Paired t-tests and Wilcoxon non-parametric tests were conducted to compare the outcome measures before and after dietary intervention. In the post-hoc analysis comparing the characteristics of Response (R) versus Non-response (NR) groups, parametric t-tests and non-parametric Mann-Whitney U tests were used to compare continuous variables, while categorical variables were compared using Chi-squared test. A two-tailed p-value <0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 25.

2.1.4 Results

2.1.4.1 Analysis of research subject characteristics
The research subjects all had a diagnosis of ASD and their ages ranged from 3 to 14 years with a mean age of 5.7 (SD ±2.9). The ratio of boys to girls in this study was 52:8, slightly higher than the ratio found in population prevalence surveys (see Chapter 1 section 1.2). The characteristics of the research subjects is shown in

Table 2-2 Not all data items were recorded for all research subjects. A record of co-occurring conditions is available for 58 research subjects and is summarised in

Table 2-3. These were frequently reported and diverse, with GI symptoms, developmental regression and sleeping problems being the most frequently reported symptoms. 25% of research subjects with data recorded on the presence or absence of sleeping problems (n=14), reported more than one sleeping problem. Of the 31 research subjects with allergy, 17 of these had allergies to multiple agents.

Table 2-2: Characteristics of study participants

Characteristic	Number	Ratio or percentage	Number of participants for the data item
Sex male; female	52; 8	13:2	n=60
Type of delivery VD; VDI; CS	16; 13; 17	-	n=46
Age at first appointment (years) (mean±SD)	5.7±2.9	-	n=60
Period of breastfeeding (months) (mean±SD)	7.61±8.23	-	n=43
Autoimmune history in the family	20	47%	n=43
Maternal antibiotic use in pregnancy or lactation (number, %)	11	28%	n=39
Child taking a probiotic prior to intervention (number, %)	17	40%	n=42
History of antibiotic use in participant (number, %)	35	73%	n=48
Subject had >3 courses of antibiotics	22	46%	n=48

Note: VD= vaginal delivery; VDI= vaginal delivery with instrument; CS= C-section

Table 2-3: Prevalence of co-occurring conditions

Co-occurring condition	Number with condition	Percentage with condition	Number of subjects with the data item completed
GI symptoms	47	81%	58
Constipation	31	54%	58
Diarrhoea	24	41%	58
Abdominal pain	16	28%	58
Sleeping problems	40	70%	57
Difficulty falling asleep	20	35%	57
Disturbed sleep[a]	29	51%	57
Developmental regression	38	75%	51
Allergy	31	61%	51
Atopy	23	33%	59
Joint hypermobility	21	35%	60
Anxiety	19	53%	36
Ear infection	15	26%	57

[a]Includes night waking and waking early in the morning

2.1.4.2 Assessment of adherence to personalised diet interventions

The clinical records of 21 research subjects were chosen at random and were assessed for diet and supplement programme adherence. At follow-up appointments parents completed a 1-day diet diary for their child and a list of nutritional supplements being given with dosages. If they had implemented between 20 – 80% of the recommendations from the previous appointment, adherence was graded as Fair; less than 20% was graded Poor; and greater than 80% was graded as Good. Data on nutritional supplement adherence was only available for 19 of the 21 research subjects

chosen. For the personalised diet programmes, 86% achieved either good or fair adherence. For the personalised supplement programmes, 79% achieved either good or fair adherence.

2.1.4.3 Efficacy assessment of dietary intervention using ATEC

For all those with complete ATEC data (n=29), the mean ATEC Total score significantly improved, 69.9 ±29.4 (Mean ±SD) compared to 56.4 ±24.9, p<0.0001. This result suggests that dietary intervention may improve global wellbeing and function in autistic children. All the mean ATEC subscale scores reduced with diet intervention as shown in Table 2-4 and Figure 2-1.

Table 2-4: ATEC scores before and after treatment

ATEC Assessment	Mean baseline score (SD)	Mean score after treatment (SD)	Mean difference (SD)	95% CI		Paired T-test p-value	Wilcoxon p-value
				Lower	Upper		
Total	69.9 (±29.4)	56.4 (±24.9)	13.5 (±14.9)	7.8	19.2	<0.001	0.000
HPB	27.5 (±11.4)	21.0 (±11.9)	6.3 (±8.8)	2.7	10.0	0.002	0.003
SLC	13.4 (±7.7)	11.1 (±7.4)	2.8 (±2.8)	1.7	4.0	<0.001	0.000
Social	14.6 (±6.7)	11.0 (±6.2)	3.2 (±5.2)	1.0	5.4	0.006	0.004
SCA	16.6 (±8.5)	14.5 (±8.0)	1.9 (±7.4)	-1.1	5.0	0.206	0.013

Abbreviations: CI confidence interval; SD standard deviation; HPB Health, Physical, Behaviour; SLC Speech, Language, Communication; SCA Sensory, Cognitive Awareness; Social Sociability.

Figure 2-1: Mean ATEC scores before and after treatment

Mean ATEC results before and after treatment
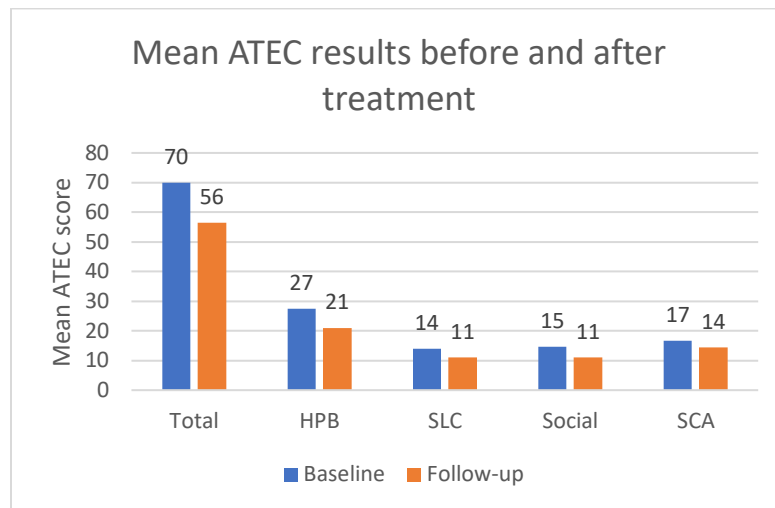
Abbreviations: HPB Health, Physical, Behaviour; SLC Speech, Language, Communication; SCA Sensory, Cognitive Awareness; Social Sociability.

2.1.4.4 Buie as efficacy assessment

For those with complete Buie data (n=29), the mean Buie score decreased significantly from 23.5 ±12.8 (Mean ±SD) to 19.7 ±13.1 (Mean ±SD) (p=0.011). Although this may be indicative of a reduction in the frequency or severity of abdominal pain, this is an unvalidated measure.

2.1.4.5 Organic acid test as efficacy assessment

There was complete Organic Acid data for 18 research subjects. Analysis of the data for these subjects showed a positive effect following dietary intervention. The mean 4-Hydroxyphenylacetic acid level reduced from 22.8 ±19.1(Mean ±SD) to 14.7 ±7.0, p=0.077.   The mean HPHPA level reduced from 138.0 ±86.7 (Mean ±SD) to 61.8 ±77.3, p<0.001. Although this is a relatively small sample size, it suggests that dietary intervention may be helpful as an adjunct to treatment for clostridia overgrowth and small intestinal bacterial overgrowth.

2.1.5 Post-hoc responder analysis

2.1.5.1 Post-hoc responder analysis based on ATEC total score

The assessment results showed a positive effect for personalised dietary intervention for autistic children but there were large differences between individuals, suggesting the presence of a subgroup who are more likely to benefit from this intervention. The

29 research subjects with baseline and follow-up ATEC data were divided into Response (R) (n=18) and Non-response (NR) (n=11), (as defined in section 2.1.2). The mean baseline and follow-up scores of Response and Non-response groups are given in Table 2-5. An analysis of the change in mean ATEC scores after dietary intervention between these two groups is given in Table 2-6 and Figure 2-2. The mean change in Total ATEC score from baseline to after treatment for the Response vs Non-response groups is   -22.8 ±9.9 (mean ±SD) and 1.7 ±6.8 (mean ±SD), respectively P=1.8823E-8.   The mean change in three of the ATEC subsection scores from baseline to after treatment showed a significant difference between Response and Non-response groups: These were HPB -10.0 ±4.9 (mean ±SD) vs 0.2 ±10.6 P=0.0212; Sociability -4.8 ±5.7 (mean ±SD) vs -0.6 ±2.9, P=0.024 and SCA -5.3 ±3.6 (mean ±SD)   vs 3.1 ±8.9,   P=0.016. Speech, Language and Communication subsection score showed an improvement (reduction) in both groups but the difference between the two groups did not reach statistical significance.

Table 2-5: Mean ATEC scores before and after treatment for Response (R) and Non-response (NR) groups

ATEC measure	R mean baseline score (SD)	R mean score after treatment (SD)	NR mean baseline score (SD)	NR mean score after treatment (SD)
Total	82.2 (±21.5)	59.4 (±22.7)	49.8 (±30.5)	51.5 (±28.6)
HPB	31.2 (±7.5)	21.2 (±10.5)	20.9 (±14.4)	21.1 (±15.2)
SLC	16.6 (±7.7)	13.2 (±7.2)	10.0 (±6.6)	8.1 (±7.1)
Social	17.5 (±5.5)	12.7 (±7.0)	9.0 (±4.9)	8.4 (±4.1)
SCA	20.3 (±6.5)	15.0 (±6.6)	11.0 (±8.8)	14.1 (±10.4)

Note: Response group, n=18 for Total, n=16 for HPB and n=15 for other subsection scores; Non-response group, n=11 for Total, n=10 for SCA and n=9 for other subsection scores.

Table 2-6: Analysis of mean change in ATEC scores after treatment between Response (R) and Non-response (W) groups

ATEC Assessment	R mean change in score (SD)	NR mean change in score (SD)	Mean diff. between groups	Equal var. assumed	95% CI		T-test p-value	Mann Whitney Significance
					Lower	Upper		
Total	-22.8 (±9.9)	1.7 (±6.8)	-24.5	No	-30.9	-18.1	1.8823E-8[**]	5.781E-8[1][**]
HPB	-10.0 (±4.9)	0.2 (±10.6)	-10.2	No	-18.6	-1.9	0.0212[*]	0.001[**]
SLC	-3.4 (±2.9)	-1.9 (±2.4)	-1.5	No	-3.8	0.8	0.182	0.263
Social	-4.8 (±5.7)	-0.6 (±2.9)	-4.2	No	-7.9	-0.6	0.024[*]	0.15
SCA	-5.3 (±3.6)	3.1 (±8.9)	-8.4	No	-14.9	-1.9	0.016[*]	1.646E-8[1][**]

Notes: Response group, n=18 for Total, n=16 for HPB and n=15 for other subsection scores; Non-response group n=11 for Total, n=10 for SCA and n=9 for other subsection scores. Abbreviations: diff. difference, var. variance

[**] statistically significant at p<0.001, [*] statistically significant at p<0.05

The fact that there are statistically significant differences in the mean change in the two subsection scores for Health Physical Behaviour and also Sensory Cognitive Awareness, suggests that the effect of the personalised diet intervention is broader than simple improving physical health symptoms such as constipation and diarrhoea.

Figure 2-2: Comparison of mean change in ATEC scores between Response and Non-response groups
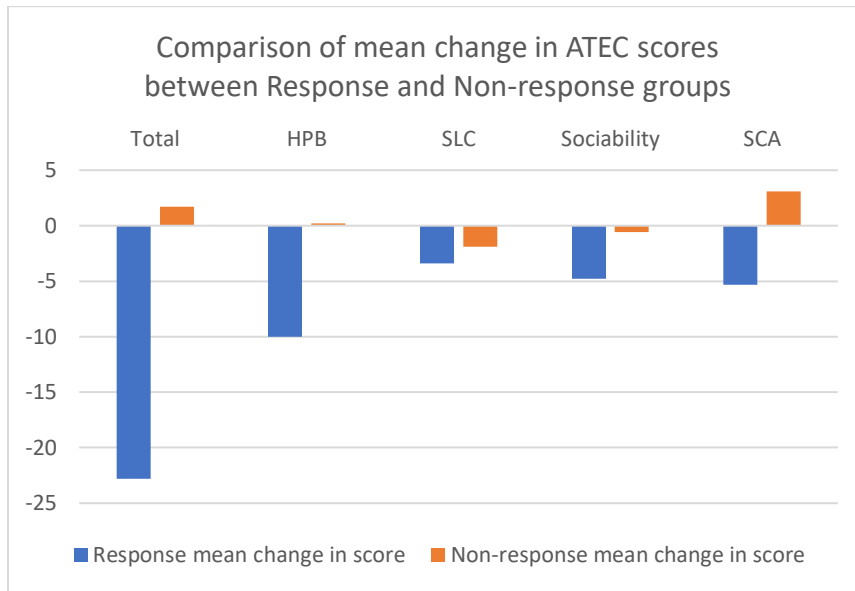
Note: a reduction in ATEC score indicates an improvement in the participant

Comparative analysis using parametric and non-parametric analysis was completed on the characteristics of the Response and Non-response group to determine which factors were indicative of response to dietary intervention in autistic children. The children in the Response group had higher baseline ATEC scores and this applied to the Total score 82.2 ±21.5 (mean ±SD) vs 49.8 ±30.5, p=0.007 and two subsection scores, Sociability 17.8 ±5.4 (mean ±SD) vs 9.0 ±4.9, p=0.001; and Sensory/Cognitive/Awareness 20.1 ±6.3 (mean ±SD) vs 11.0 ±8.8, p=0.005 as shown in Table 2-7 and Figure 2-3. ATEC Speech/Language/Communication was approaching statistical significance in parametric analysis and a larger sample size may find a difference between the Response and Non-response groups. Considering other continuous variables, the Response group had a lower average age when starting dietary intervention; a shorter period of breastfeeding; and a higher age of first antibiotic use but none of these were statistically significant (see Table 2-7). Other baseline and follow-up assessment results (Buie score, HPHPA and 4-Hydroxyphenylacetic) did not reveal significant differences between the Response and Non-response groups. The after-treatment 4-Hydroxyphenylacetic nearly reached statistical significance in non-parametric analysis despite the small number of participant results and a larger sample size might reveal a significance.

Table 2-7: Comparison of continuous variables between ATEC Response and Non-response

					95% CI		
Variable	ATEC Response mean±SD (N)	ATEC Non-response mean±SD (N)	Equ. var.	t-test P-value	Lower	Upper	Mann-Whitney U Sig.
Age starting dietary intervention	5.0±1.7 (17)	5.4±1.6 (7)	yes	0.631	-1.9	1.2	0.619
Months of breast-feeding	6.8±5.5 (14)	10.6±13.3 (9)	no	0.443	-14.2	6.7	0.975
Age of first antibiotic[a]	0.87±1.2 (9)	0.80±0.5 (6)	yes	0.906	-1.1	1.27	0.607
Baseline assessment							
ATEC total	82.2 ±21.5 (18)	49.8 ±30.5 (11)	no	0.007*	10.2	54.6	0.009*
ATEC HPB	31.2 ±7.5 (16)	20.9 ±14.4 (9)	no	0.073	-1.1	21.7	0.095
ATEC SLC	16.2 ±7.6 (16)	10.0 ±6.6 (9)	yes	0.053^	-0.09	12.5	0.074
ATEC Social	17.8 ±5.4 (16)	9.0 ±4.9 (9)	yes	0.001*	4.2	13.3	0.001*
ATEC SCA	20.1 ±6.3 (16)	11.0 ±8.8 (10)	yes	0.005*	3.0	15.2	0.010*
Buie	27.5±10.7 (11)	19.8±12.9 (8)	yes	0.168	-3.6	19.2	0.109
HPHPA	200.7±184.5 (16)	110.4±81.0 (9)	yes	0.180	-44.6	225.2	0.187
4-HPAA	20.7±11.9 (16)	22.0±24.0 (9)	yes	0.859	-16.0	13.5	0.357

Follow-up assessment							
ATEC total	59.4 ±22.7 (18)	51.5 ±28.6 (11)	yes	0.417	-11.8	27.6	0.492
ATEC HPB	21.0 ±10.2 (17)	21.1 ±15.2 (9)	yes	0.982	-10.4	10.2	0.367
ATEC SLC	12.8 ±7.1 (16)	8.1 ±7.1 (9)	yes	0.128	-1.5	10.9	0.108
ATEC Social	12.4 ±6.9 (16)	8.4 ±4.1 (9)	yes	0.127	-1.2	9.2	0.136
ATEC SCA	14.7 ±6.5 (16)	14.1 ±10.4 (10)	yes	0.860	-6.2	7.4	0.586
Buie	22.9±15.0 (15)	19.6±11.6 (10)	yes	0.558	-8.3	14.9	0.567
HPHPA	70.5±116.2 (6)	83.1±55.6 (7)	yes	0.802	-121.0	95.7	0.234
4-HPAA	11.4±4.5 (6)	24.6±14.5 (7)	yes	0.056	-26.8	0.4	0.051^

Notes: [a] in years; * $p<0.05$; ^ approaching statistical significance. Abbreviations: Equa. var. equal variance assumed

Categorical variables were also subjected to comparative analysis between the two groups using Chi-squared analysis (Table 2-8) but none of these variables showed an association with the research subject's response to dietary intervention. However, it is worth noting that research subjects in the response group had a higher incidence of constipation and a lower incidence of allergies and a larger sample size may show an association between these and treatment response.

Figure 2-3: Comparison of mean baseline ATEC scores, Response vs Non-response groups

Mean baseline ATEC scores
Response vs Non-response
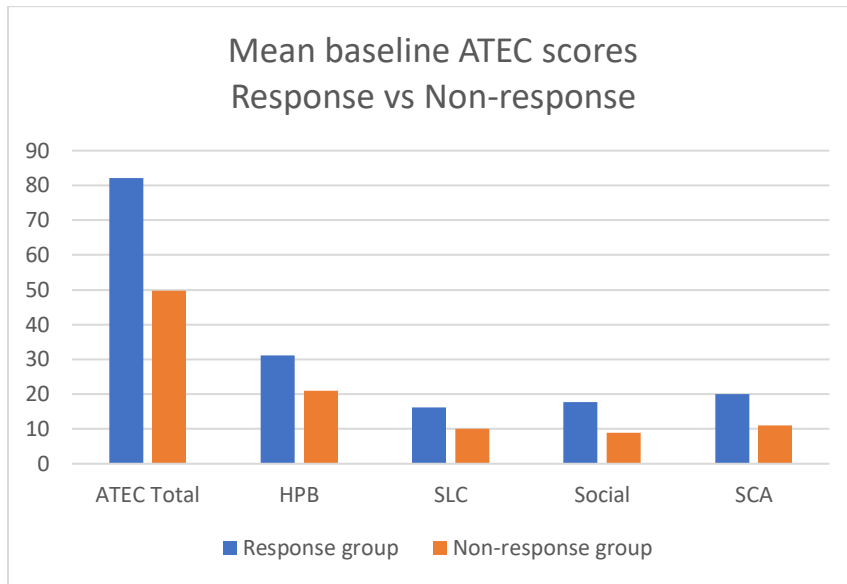
Table 2-8: Comparison of categorical variables, Response vs Non-response groups

Variable	Response group (N*)	Non-response group (N)	Asymp. Sig
Allergies	53% (15)	80% (10)	0.174
Constipation	67% (15)	30% (10)	0.072
Diarrhoea	56% (16)	56% (9)	0.973
Abdominal pain	45% (11)	29% (7)	0.474
Anxiety	50% (10)	50% (6)	1.0
Hypermobile	50% (10)	80% (5)	0.264
Sleep problems	65% (17)	70% (10)	0.778
Use of antibiotics	86% (14)	90% (10)	0.754

*N: Available data in each characteristic

2.1.5.2 Post-hoc responder analysis based on Buie score

The Buie assessment is a clinical tool used to evaluate and monitor the frequency and severity of GI distress in autistic children. For the 29 research subjects with complete

Buie data, the subjects were split into Buie response (n=14) and Buie Non-response (n=15) on the basis described in section 2.1.2.

For the Buie Response group (BR), the mean change in Buie score from baseline to after treatment was -9.21 ±5.2 (mean ±SD). For the Buie Non-response group (BNR), the mean change in Buie score was 1.2 ±5.6 (mean ±SD). The mean difference of the post-treatment change in Buie scores between BR and BNR groups was -10.5 p=0.00002, (95% CI -14.6 to -6.3, equal variances assumed) indicating a significantly different response to dietary intervention between the two groups. Non-parametric independent samples Mann-Whitney U test also showed statistical significance p=2.579E-8[1] for the mean change in Buie score following treatment comparing the BR and BNR groups.

An analysis of characteristics of the Buie Response (BR) group vs Buie Non-response (BNR) group was completed to explore factors that differ between the two groups. Continuous variables were analysed using parametric and non-parametric tests with results shown in Table 2-9. This showed significant differences between the BR and BNR groups in baseline scores for ATEC HPB (mean difference 12.0, p=.013), ATEC Sociability (mean difference 6.7, p=.038), ATEC SCA (mean difference 7.8, p=.044), with all three scores being higher in the BR group compared to the BNR group. However, there was no significant difference between the two groups in baseline ATEC Total score (mean difference 14.4, p=.30). This suggests that personalised dietary intervention is more likely to reduce gastrointestinal distress (subject to the validity of the Buie assessment) in those autistic children that have higher scores in the ATEC subscales of HPB, Sociability and SCA.

Following treatment, there were no significant differences between the BR and BNR groups in any of the ATEC subscale scores: this suggests that reducing GI distress is not impacting one particular subscale of the ATEC. However, the difference in ATEC Total score between the two groups approaches statistical significance (mean difference 19.3, p=.059) and given the small sample size, may be significant in a larger sample. This suggests that reducing GI discomfort is improving overall wellbeing and function. The baseline Buie score did not show a significant difference between the BR and BNR group. This indicates that it is not just the children with notable GI

distress that are reporting a reduction in GI distress after personalised diet intervention.

Table 2-9: Comparison of continuous variables for the Buie Response (BR) and Buie Non-response (BNR) groups

Variable	BR group (N)	BNR group (N)	Equal var.	t-test P-value	95% CI Lower	Upper	Mann-Whitney U Sig.
Age starting treatment	5.4 ±12.9 (13)	5.1 ±1.9 (13)	yes	0.797	-1.76	2.27	0.840
Months of breast-feeding	6.5 ±5.6 (11)	12.2±11.6 (13)	no	0.136	-13.3	1.96	0.331
Age of first antibiotic[a]	0.85 ±1.2 (10)	0.46 ±0.5 (10)	yes	0.359	-0.48	1.26	0.481
Baseline assessment							
Buie	26.6±11.2 (14)	20.6±13.8 (15)	yes	0.209	-3.6	15.7	0.158
ATEC total	68.2 ±36.8 (11)	53.8 ±28.2 (12)	yes	0.301	-13.8	42.7	0.260
ATEC HPB	31.8 ±5.7 (8)	19.7 ±12.5 (11)	no	0.013[*]	2.87	21.2	0.091
ATEC SLC	14.8 ±8.1 (8)	11.4 ±7.4 (11)	yes	0.356	-4.14	10.9	0.310
ATEC Social	17.4 ±5.2 (8)	10.7 ±7.1 (11)	yes	0.038*	0.4	12.9	0.041[*]
ATEC SCA	19.3 ±7.4 (9)	11.5 ±8.4 (11)	yes	0.044*	0.24	15.3	0.080

HPHPA	218.0±223.1 (10)	126.0±66.0 (14)	no	0.234	-69.7	253.7	0.312
4-HPAA	19.8±13.5 (10)	22.4±19.1 (14)	yes	0.718	-17.2	12.0	0.666
Post-treatment assessment							
Buie	17.4±10.1 (14)	21.9±15.4 (15)	no	0.371	-14.3	5.5	0.715
ATEC total	71.7 ±27.2 (12)	52.4 ±21.2 (13)	yes	0.059$^\wedge$	-0.78	39.3	0.087
ATEC HPB	25.4 ±12.7 (12)	18.5 ±8.7 (12)	yes	0.133	-2.3	16.1	0.128
ATEC SLC	14.7 ±8.1 (11)	9.3 ±6.3 (12)	yes	0.087	-0.85	11.6	0.104
ATEC Social	15.1 ±6.9 (11)	11.1 ±7.5 (12)	yes	0.197	-2.25	10.3	0.079
ATEC SCA	17.5 ±7.6 (11)	12.8 ±9.2 (12)	yes	0.197	-2.6	12.1	0.151
HPHPA	88.2 ±87.7 (5)	60.4±59.8 (9)	yes	0.492	-57.7	113.3	0.518
4-HPAA	17.2±4.5 (5)	18.8±14.0 (9)	yes	0.816	-15.8	12.7	0.438

Notes: [a] in years; [*] $p < 0.05$; [^] approaching statistical significance

In the non-parametric analysis only the baseline ATEC Sociability score showed a significant difference between the two groups.

Categorical variables were also subjected to comparative analysis between the two groups using Chi-squared analysis (

Table 2-10). The only significant difference was a higher proportion of research subjects with allergies at baseline in the Buie Non-response group.

Table 2-10: Comparison of categorical variables for Buie Response (BR) and Buie Non-response (BNR) groups

Variable	BR group (N)	BNR group (N)	Asymp. Sig
Allergies	50% (12)	86% (14)	0.049*
Constipation	54% (13)	43% (14)	0.568
Diarrhoea	73% (11)	36% (14)	0.066
Abdominal pain	55% (11)	33% (12)	0.305
Anxiety	57% (7)	64% (11)	0.783
Hypermobile	50% (8)	60% (10)	0.671
Sleep problems	79% (14)	67% (15)	0.474
Use of antibiotics	83% (12)	77% (13)	0.689

N: Available data in each characteristic; * statistically significant at p<0.05

2.1.6 Discussion

2.1.6.1 ATEC – validity and limitations

The hypothesis being explored in this thesis is whether dietary management of GI symptoms can improve overall wellbeing and function in autistic children. Consequently, a global measure of function was required that was sensitive to change and designed to assess the effect of an intervention over time in autistic children. There are a large number of outcome measures that have been used for assessing the effect of a treatment or intervention in autistic children [192]. In a 2015 systematic review of assessment tools for measuring treatment or intervention outcome in young autistic children, it was concluded that there was a lack of fully robust tools for this group, so there will be limitations to any chosen assessment tool. In that same review, the ATEC was reviewed as one of only four measures of global function, and was the only one of the four with evidence of responsiveness to change [192]. The ATEC is completed by a parent, care-giver or educator and provides a total score and four sub-section scores in the categories of Speech, Language and Communication (SLC);

Sociability (Social); Sensory, cognitive, awareness (SCA); and Health, Physical and Behaviour (HPB): A higher score indicates lower wellbeing and a greater impact of autistic traits. The ATEC total score ranges from 0-180 and is calculated by adding the sub-section scores together.

The ATEC has been successfully used in a number of studies to measure the effectiveness of interventions for autistic children or to measure the impact of autistic traits [193][194][195][196][197][58][198][199][200]. It has been validated against the Childhood Autism Rating Scale (CARS), which is validated and well-established scale for the impact of autistic traits [201]. Some autism diagnosis measures have been used in research studies to evaluate intervention effectiveness (Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule and CARS) but these measures have been designed to show stability over time and are not sensitive to change. The ATEC is unique as it not only assesses the impact of autistic traits but also evaluates the physical issues often present in autistic children. The ATEC was examined for its usefulness in monitoring the progress of autistic children and it was concluded that it is potentially a valid measure of change in overall function over time [202]. This study included 22 autistic boys aged between 5-6 years of age but since no girls were included in the sample, it is unclear whether this finding would apply to autistic girls. A possible limitation in sensitivity in the ATEC has been found for autistic children with near age-appropriate language skills and therefore it may be more appropriate for monitoring the progress of autistic children with moderate to severe traits [203]. Standard assessments that have been developed for non-autistic children are inappropriate for monitoring the progress of autistic children as their development pattern is very different, with the added complication that it varies considerably from one autistic child to another [203]. The ATEC shows high internal consistency with the total and subscale scores remaining reasonably stable over time and the total and subscale scores correlate significantly with other standard instruments (Expressive One-Word Picture Vocabulary Test, CARS, Vineland Adaptive Behaviour Scales, ADI-R, British Picture Vocabulary Scales,) [201,203]. It is often questioned whether parents can objectively and accurately assess their child, but there is evidence that parents of autistic children can be reliable in assessing their child's behavior and functioning when compared to assessments done by professionals [204] [203][205].

Indeed, where a child has a developmental delay, even clinician-rated assessments rely on parents as informants [206].

Unlike ADOS, CARS and Mullen Scale of Early Learning (MSEL), norms have been estimated for the ATEC total and subscale scores to provide a reference for caregivers to assess the impact of their child's autistic traits, track development and project future impact of autistic traits. The calculation of the norms was done using a dataset of 2649 participants, 83% male and 17% female, aged 1.5 – 12.5 years old. The ATEC assessments were all completed online so there is no confirmation of an ASD diagnosis. Participation was voluntary and participants were international. The children were participating in various interventions, so the norms do not reflect progress without intervention. The researchers excluded those with a total ATEC score of less than 20 to try and avoid including non-autistic children but the inadvertent inclusion of some non-autistic children may over-estimate the improvement. They found no difference in the improvement between girls and boys, but those from English speaking developed countries had less improvement over time. Those with an ATEC total score of greater than 70 at 2 years, improved significantly but plateaued at age 12, with the plateaued score being proportional to the score at age 2 years. Those with an ATEC score of less than 70 at 2 years, improved initially but then demonstrated a deterioration in the ATEC total and subscale scores (except Health and Physical Behaviour), after 7 years of age. The authors hypothesis that this reflects a change in parents attitude to certain behaviours assessed by the ATEC which they report as more of a problem at 7 years or older compared to when the child is 2 years old [205]. In 2012 the ATEC was reviewed along with 16 other instruments for its appropriateness in measuring social communication behaviour in autistic children participating in clinical trials. Insufficient evidence was found to support using the ATEC for this. One of the reasons was its limited sensitivity to change for social communication behaviour, therefore limited change in the Sociability subscale of the ATEC should be expected following intervention.

2.1.6.2 Limitations

This is a real-world study using pre-existing clinical data and suffers the inherent limitations of such a study. The data was extracted from a clinical database and suffers from incomplete data and unrecorded confounding factors such as other

concurrent therapies. The parents had chosen to bring their child to the clinic and were paying for the service, so there may to be selection bias, and parents may have been anticipating an improvement in their child. Both the ATEC and Buie are parent-completed assessments and although parents have been shown to be comparable with health professionals when reporting their child's global function and GI symptoms [197] [202] [148], it has also been shown that they report a strong placebo effect [207]. This is an uncontrolled, open study and therefore there is no possibility of assessing the placebo effect. The study benefits from a reasonable sample size, and the effectiveness assessment measured by the ATEC has strong statistical significance, however given the limitations, these results should be regarded with caution. Further large prospective studies are needed to determine the effectiveness of personalised diet intervention for autistic children in improving global wellbeing and function.

2.1.6.3 Summary

In summary, this analysis of pre-exisiting clinical data from 60 autistic children participating in routine personalised dietary intervention, indicates that dietary intervention may improve overall wellbeing and function in autistic children. There was a wide variation in response to treatment and there are insights we can gain about the possible mechanisms and who might benefit, from the results of the two post-hoc responder analyses performed. The first responder analysis based on change in ATEC Total score, indicated that those starting with a higher ATEC Total score or a higher subscale score for Sociability or Sensory/Cognitive/Awareness are more likely to respond to dietary intervention. This responder analysis showed statistically significant differences in the mean change for the two subscale scores for Health Physical Behaviour and also Sensory Cognitive Awareness: This suggests that the effect of the personalised diet intervention is broader than simply improving physical health symptoms such as constipation and diarrhoea.

The second responder analysis was based on the change in Buie score which is an unvalidated assessment of possible abdominal pain in autistic children. Those in the responder group in this analysis reported an improvement in symptoms of GI distress (subject to the validity of the Buie assessment). A post-hoc analysis of the characteristics of these responders compared to non-responders indicates that personalised dietary intervention is more likely to reduce GI distress in those autistic

children that start with higher scores in the ATEC subscales of HPB, Sociability and SCA. Following personalised diet intervention, there were no significant differences between the responder and non-responder groups in any of the ATEC subscale scores: this suggests that reducing GI distress is not impacting one particular subscale of the ATEC. In line with this, the difference in ATEC Total score between the responder and non-responder groups approaches statistical significance suggesting that reducing GI discomfort is improving overall wellbeing and function. There was not a significant difference in the starting Buie score between the responder and non-responder groups, which indicates that it is not just children with notable GI distress that are reporting a reduction in GI distress after personalised diet intervention.

However, it is limited study and we should be cautious about drawing conclusions. To help understand the possible mechanisms of personalised dietary intervention and who may benefit from which diet interventions, the next step was to undertake a more targeted intervention, in a more targeted sample group which led to the Vivomixx clinical trial documented in Chapter 3.

Chapter 3   Vivomixx clinical trial

VIVO-ASD STUDY, IRAS 204582, UCL JRO 17/0148

This study evaluates the efficacy of Vivomixx probiotic to improve global function and gastrointestinal symptoms in autistic children. It is a randomised, double-blind, placebo-controlled crossover trial of Vivomixx probiotic for autistic children with persistent gastrointestinal symptoms.

3.1   Rationale for the study

In the analysis of my clinic data presented in Chapter 2, a probiotic was the most common intervention being recommended to 70% of the research subjects so this became the focus for a more targeted dietary intervention. There is some evidence that probiotics can improve GI symptoms in autistic children and also bring wider benefit. The majority of the published studies in this field are methodologically weak and the results needed to be confirmed with a double-blind randomised controlled study. Parents of children with disabilities are known to suffer increased parental stress, particularly parents of autistic children [208], so any treatment should be assessed considering the burden on parents. Giving a probiotic is a low-risk, easy intervention that should not over-burden parents of autistic children.

The over-the-counter supply of probiotics can be confusing for the consumer with different formats (liquid, capsules, powder); a wide variety of brands, probiotic species and strains; varying quantities of probiotic per dose; and different instructions on how to take the probiotic (before food, with food). The only NICE guidelines on probiotics are for irritable bowel syndrome (IBS) patients and the guidelines suggest that if IBS patients choose to try probiotics, they should take the probiotic product at the manufacturer-recommended dose for a period of at least 4 weeks and self-monitor the effect. It is likely that consumers' probiotics choices will vary according to their finance, personal experience, recommendations from people they trust; and preference for format. In a UK survey of parents of autistic individuals [47], 90% of respondents had given a food supplement to an autistic person in their care and probiotics were one of the food supplements often given. This level of use warrants the pursuit of robust evidence to guide health professionals and parents in their choice of probiotic for the

autistic individual in their care. To try and address this gap in the research, I conducted a randomised double-blind placebo-controlled crossover trial of Vivomixx probiotic for autistic children with persistent GI symptoms.

3.2   The study design

The study design was developed in consultation with parents of autistic children. The draft study design was reviewed by the London Autism Research Advisory Group which is a parent-led group. A taste evaluation of the product was included in Week 1 following concerns from parents about sensory sensitivities of autistic children.

There is considerable heterogeneity amongst those with an autism diagnosis and finding suitable controls for participants is therefore very difficult. To overcome this, the study was designed as a crossover study so that each participant became their own control. Another advantage of this design is that all participants have the opportunity to access the active treatment. A potential downside of the crossover design is that there may be a carry-over effect where the probiotic is the first treatment. To protect against this there was a 4-week break between the two treatment phases to allow the effects of Part 1 treatment to wash out before starting Part 2 treatment. Most crossover clinical trials use a washout period of between 2 and 4 weeks and the developer of Vivomixx advised that the product can take a little over 2 weeks to wash out.

The subgroup of autistic children with persistent GI symptoms was chosen because it was believed that they are more likely to have a positive response to Vivomixx given its success for children with Irritable Bowel Syndrome (IBS) [209]. Vivomixx probiotic was chosen because it was formulated for inflammatory bowel disease (IBD) and Crohn's disease and has been extensively studied (under the brand name of VSL3). It's a high-dose, multi-species formula that has been studied in children with IBS [209] and is available on the open market. A 12-week course of treatment was chosen for the treatment phases as it allows time for good colonization of the gut and is sufficiently long to determine clinically significant changes in GI symptoms and behaviour.

3.2.1 Outcome measures

3.2.1.1 Primary outcome measure

The percentage change in the Autism Treatment Evaluation Checklist (ATEC) Total score from T0 to after Vivomixx compared to after placebo. The ATEC was chosen because it is global measure of function in autistic children that is sensitive to change (see 2.1.6.1 for details on the ATEC). The total score ranges from 0 – 180 and is calculated by the summation of the four subscale scores. A higher score indicates greater challenges for the child and more pronounced autism traits.

3.2.1.2 Secondary outcome measures

1. The change in frequency of GI symptoms from T0 following Vivomixx compared to placebo.

A secondary outcome of this study are the frequency scores of the participant's GI symptoms assessed using the Gastrointestinal History questionnaire (GIH). The GIH was developed in 2003 [210] specifically for autistic children. It includes 10 Likert scale items for the frequency of: abdominal pain, constipation, pain on defecation, gaseousness/bloating, diarrhoea, vomiting, sensitivity to foods, difficulty swallowing, blood in stools and blood in vomit. There are four yes/no questions about the presence of food allergies and intolerances, special diets followed, diet restrictions due to food dislikes, and GI disease diagnosis. Three open-ended questions collect information on food allergies; the reasons for any diet restrictions; and any GI conditions that have been diagnosed. In this study, the GIH was used in conjunction with the Bristol Stool Scale illustration as a pro-forma for interview with the participant and their primary carer.

2. The change in Aberrant Behaviour Checklist (ABC) section scores from T0 following Vivomixx compared to placebo.

Behavior was assessed using five section scores of the ABC: Hyperactivity/Noncompliance; Irritability/Agitation/Crying; Stereotypic behaviour; Lethargy/Social Withdrawal; and Inappropriate Speech as validated by Kaat et al [211]. The higher the section score, the greater the behaviour is rated as problematic by the Primary Carer or educator. This is a 58 item, parent or educator completed assessment that is designed to measure the effect of interventions and has been used

extensively in clinical trials targeted at autistic children and those with other developmental disorders. It has been proven to have excellent reliability [211–213]. In 2012, the Social Withdrawal subscale of the ABC was identified by a review as having the strongest evidence for use as an outcome measure in clinical trials when measuring the social domain of autism, where the ATEC is weak.

3. The change in ATEC subscale scores from T0 following Vivomixx compared to placebo.

The percentage change in the ATEC subscale scores from T0 to after Vivomixx compared to after placebo.

4. The change in Autism Parenting Stress Index (APSI) from T0 following probiotic treatment compared to placebo.

A secondary outcome is the change in the APSI score from T0 following Vivomixx compared to placebo. This parent-completed questionnaire was developed and validated in 2012 [214]. It assesses parent stress regarding certain aspects of autism that can concern parents. It is designed to measure a change in parenting stress following an intervention. Parents rate thirteen aspects of their child's wellbeing on a 0 – 5 scale on the basis of how stressful it is for them and/or their family. The APSI total score ranges from 0 – 65 with a higher score indicating more parenting stress. The APSI has been successfully used as an outcome measure in a study assessing the efficacy of a massage therapy for autistic children [215].

5. The change in Clinical Global Impression from T0 following Vivomixx compared to placebo.

Based on discussion with the child and parent, and behaviour observed in the appointment, a clinical global impression score was given for gastrointestinal symptoms and behaviour, on a 0 to 100 scale, with 0 being no symptoms and 100 being the worst possible.

6. Parent and researcher assessment of treatment effectiveness

After Vivomixx and after placebo the child's primary caregiver was asked to score the effectiveness of the treatment on a scale of 0 to 10, with 0 being no change and 10 being the best they would expect.

3.2.2 Sample size calculation

The recruitment target for this study was calculated using a sample size calculation. It was calculated that a sample size of 72 would be sufficient to determine an effect size of 0.50 with 80% power and a type 1 error of 5% (two-sided test). In the case of a 15% drop-out rate this would mean that 82 participants are needed. This calculation was based on the assumed effect size of the primary outcome measure (ATEC Total score). The minimum clinically significant change based on the ATEC Total score was taken as 15 points, which seemed a reasonable assumption considering previous probiotic efficacy data. Previous studies using the ATEC Total have indicated that it is reasonable to assume that the data will follow a normal distribution. Considering a 10% drop-out rate, the sample size required is reduced to 74. With a type 1 error rate of 5%, and a power of 80%, a non-parametric matched pairs analysis (Wilcoxon Signed-Rank Test) and assuming a low correlation between the subjects in the two conditions (rho=0.2), the statistics programme G*Power 3.1.7 calculated that the study would achieve 80% power with a Cohen's dz effect size of 0.34. This is equal to a small to medium Cohen's standard effect size of 0.37 (adjusted using the correction given in Myers, Well, & Lorch (2010) on page 147 equation 6.28) [216].

3.3 Methodology

A full study protocol was written and given ethical approval by the Medical Research Authority. The study is listed on ClinicalTrials.gov [217] and the overall study flow is illustrated in Figure 3-2.

A Data Monitoring Committee was established including an independent statistician and a gastroenterology consultant. This met every six months to advise on adverse events, data collection and analysis. A Trial Steering Committee was established which included three independent members, two of which were parents of autistic children. This met every 4-6 months to advise on aspects of the study and consider any recommendations from the Data Monitoring Committee.

3.3.1 Recruitment

Potential participants were recruited via autism parent support groups and charities supporting autistic children and their families. After initial email or phone contact, participants were screened for eligibility by phone. All those meeting the eligibility criteria were invited to enrol and were emailed the Participant Information sheet for parents and a separate information sheet for children.

Main Inclusion criteria:

- A diagnosis of ASD or autism using one of the standard assessment tools

- At least one persistent GI symptom for the past 6 months

- No changes in any regular medication for the last 3 months.

- Would be aged between 3 years and 16 years of age for the whole of their study involvement

Main Exclusion criteria:

- Diagnosis of Retts Syndrome, Fragile X, ulcerative colitis or Crohn's disease

- Regularly taking non-steroidal anti-inflammatories

- Antibiotics or probiotics taken in the month before enrolment

- Participated in another clinical trial in the past 3 months

292 parents expressed an interest in the study and 140 potential participants progressed to screening for eligibility, from which 69 participants were enrolled.   A programme of rolling recruitment was used which ran from February 2018 to March 2020. A partner in this study is the charity Caudwell Children, which supports families of children with a disability including autistic children. They have a large database with contact details of families they have supported and agreed to email these families about the study. The majority of recruitment was expected to come via this route. Unfortunately, the number of enrolments resulting from this mailout was poor and a major effort was then required to continually publicise the study via other charities and

groups supporting autistic children and their families, in order to maintain a steady recruitment rate.

The power calculation indicated that 72 participants were needed, and our target was 82, which allowed for a 15% drop-out rate. Recruitment was halted short of this target due to the Covid-19 lockdown. A CONSORT diagram for the study is shown in Figure 3-1. Three participants withdrew in Part 1 treatment and two participants withdrew in Part 2 treatment. Four participants were lost to follow-up in the second phase of treatment. Two participants have been classified as discontinued because the same primary carer had not completed the questionnaires throughout making them void. However, they did not drop out of the study prior to the end of Part 2 treatment so were not included in the drop-out calculation. Nine participants dropped out of the study before the end of Part 2 treatment, giving a drop-out rate of 13%.

3.3.2 Enrolment

Participants and their Primary Carer (usually a parent) visited University College Hospital London to enrol. Any questions the child or Primary Carer had about the study were answered before written consent was collected from the Primary Carer and from the child if they were over 12 years old. Participants were then given a unique Participant ID number that was used on all data collected during the study. Consenting participants were assigned to treatment groups through consecutive allocation of a subject number. The parent, educator and I were blind to which treatment was allocated first and second. Randomisation of treatment order was done by an independent contract research organisation using a 2x2 block randomisation generated electronically.

3.3.3 Taste evaluation

Prior to starting their Part 1 treatment, participants took part in a taste evaluation. All participants received the placebo product for the taste evaluation, but parents were blinded to this. The first dose was administered at University College Hospital after taking consent. Part of this process was to demonstrate to the child's Primary Carer how to mix the product into a drink. Two further doses administered at home completed the taste evaluation.

Figure 3-1; VIVO-ASD CONSORT diagram

Assessed for eligibility (n= 140)

Excluded n= 71, (Not meeting inclusion criteria n=26, Declined to participate n=45)

Randomised (n=69)

Vivomixx-Placebo n=35
Part 1 Treatment

Placebo-Vivomixx n=34
Part 1 treatment

Discontinued n=1, withdrawal by subject

Completed n=34

Completed n=32

Discontinued n=2, withdrawal by subject

Washout n=34

Washout n=32

Discontinued n=0

Completed n=34

Completed n=32

Discontinued n=0

Part 2 Treatment

Part 2 Treatment

Discontinued n=3

Completed n=31

Completed n=27

Discontinued n=5

Reasons for discontinuation
Lost to follow-up   1
Withdrawal by subject   1
Void assessments   1

Reasons for discontinuation
Lost to follow-up   3
Withdrawal by subject   1
Void assessments   1

84

3.3.4 Intervention

Vivomixx was the probiotic preparation selected for this study. Each sachet contained 450 billions of lyophilized bacterial cells belonging to eight probiotic strains;

- Streptococcus thermophilus DSM 24731

- Bifidobacterium breve DSM 24732

- Bifidobacterium longum DSM 24736

- Bifidobacterium infantis DSM 24737

- Lactobacillus acidophilus DSM 24735

- Lactobacillus plantarum DSM 24730

- Lactobacillus paracasei DSM 24733

- Lactobacillus delbrueckii subsp. bulgaricus DSM 24734

The placebo contained 2g of maltose and silicon dioxide powder and was matched to the colour, taste and texture of Vivomixx.

Dosages:

The following applied to both arms of the trial and both parts of the crossover;

- Children aged 3 – 10 years took 1 sachet of Vivomixx/placebo daily for the first 4 weeks, then following a phone call with myself, they increased the dose to 1 sachet twice daily for a further 8 weeks, provided there were no contraindications

- Children aged 11-16 years took 1 sachet of Vivomixx/placebo twice daily for the first 4 weeks, then following a phone call with myself, they increased the dose to 1 sachet 3 times daily for a further 8 weeks, provided there were no contraindications

3.3.5 Collection of study data

There were three time-points for the collection of study data; at enrolment (T0), after completing Part 1 treatment (T1) and after completing Part 2 treatment (T2).

T0

The data collected at T0 was;

- Medical history completed by primary carer
- Diet assessment completed by myself in conjunction with the child and primary carer, using visual illustrations of high-fibre foods, pulses and fermented foods
- ATEC completed by primary carer
- ATEC completed by educator
- ABC completed by primary carer
- ABC completed by educator
- GIH completed by myself in conjunction with the participant and their primary carer and using the Bristol stool scale as visual guide
- APSI completed by the primary carer
- Clinical Global Impression for GI symptoms and behaviour completed by myself

T1

The data collected at T1 was;

- ATEC completed by primary carer
- ATEC completed by educator
- ABC completed by primary carer
- ABC completed by educator
- GIH completed by myself in conjunction with the participant and their primary carer and using the Bristol stool scale as visual guide
- APSI completed by the primary carer
- Clinical Global Impression for GI symptoms and behaviour completed by myself

- Treatment effectiveness rating completed by the child's primary carer and also by myself

T2

The data collected at T2 was;

- ATEC completed by primary carer
- ATEC completed by educator
- ABC completed by primary care giver
- ABC completed by educator
- GIH completed by myself in conjunction with the participant and their primary care giver and using the Bristol stool scale as visual guide
- APSI completed by the primary care giver
- Clinical Global Impression for GI symptoms and behaviour completed by myself
- Treatment effectiveness rating completed by the child's primary care giver and also by myself

All parent and educator questionnaires were completed using standard written instructions. Excepting the medical history questionnaire, all questionnaires were completed considering the previous two weeks.

Figure 3-2; VIVO-ASD flow of study

	Taste test	12 week randomised treatment												Washout				12 week crossover tre				
Placebo	Day 1-7																					
Placebo or Probiotic		Day 8 - 76																				
No Treatment														Day 77-104								
Placebo or Probiotic																		Day 105 - 189				
Final Assessment																						
WEEK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Medical History	P																					
GI History	R													R								
Diet assessment	R																					
ATEC	PE													PE								
ABC	PE													PE								
APSI	P													P								
Global impression	R													R								
Phone interview					P R				P R												PR	
Clinic visit	P R	P R												PR								

KEY;   P=Parent/guardian, E=Educator, R=Research clinician. Abbreviations; ATEC Autism Treatment Evaluation Checklist; ABC Aberrant Behaviour Checklist; APSI Autism Parenting Stress Index; GI gastrointestinal

3.3.6 Adherence assessment and confounding factors

During the 12-week Part 1 treatment, I telephoned participants at 4-week intervals to answer questions, explain the next steps and check adherence. During these telephone calls, questions were asked about how the product was stored (refrigerated or not); what dose was being given; how many doses had been missed; whether the child was taking the whole dose; whether there had been any significant changes in the child's diet; and whether anything had changed in the child's environment that might affect their behaviour. The same questions were also asked at the T1 and T2 timepoints. All answers were recorded at the time in the Case Report Files.

3.4 Statistical Analysis

All statistical analyses were completed using IBM SPSS v27 unless overwise stated. For all null hypotheses tested, statistical significance was set at alpha = 0.05.

3.4.1 Study group characteristics

The characteristics of the study group as a whole were analysed and the descriptive statistics reported. The characteristics of the two order-of-treatment groups were compared to identify any significant differences between the two groups. Age at enrolment and ATEC Total at T0, were analysed using Levene's test for equality of variances and the t-test for equality of means. The spread of age groups in each group and the spread of ethnicity in each group were analysed using Likelihood Ratio Chi-square. Gender across the two groups was analysed using Pearson Chi-square.

3.4.2 Safety and adherence to protocol

To assess whether there were more adverse events while taking Vivomixx and whether protocol adherence was impacted, the absolute risk and relative risk when taking Vivomixx and when taking placebo were calculated for the following events: An adverse event; participant withdrawal from the study; participant lost-to-follow-up; and participant not reaching the full dose. The corresponding 95% confidence intervals (CI) were calculated manually according to published formulae. 95% confidence intervals that do not contain the value 1 are statistically significant at alpha = 0.05.

3.4.3 Intention to treat analysis

All outcome measures were tested for normal distribution using One-sample Kolmogorov-Smirnov test and Q-Q plots in order to determine whether to use non-parametric testing or T-test.

The Intention to treat (ITT) group included all participants who received at least one dose of each intervention and whose primary carer completed the outcome questionnaires at enrolment and after Part 1 treatment. Those who withdrew or were lost-to-follow-up in Part 2 (n=6) were included in the analysis and assumed to have no change in their scores from their last questionnaire assessment. Two participants who completed the study were excluded from the ITT analysis of the ATEC, and four in the analysis of the ABC, as the primary care giver had not completed the necessary questionnaires.

The null hypothesis for the primary outcome is that there is no difference between Vivomixx and placebo in the percentage change from T0 of the ATEC Total score. This was assessed using a paired T-test, 2-sided. For each of the four sub-section scores of the ATEC the null hypothesis is that there is no difference between Vivomixx and placebo in the percentage change from T0 of the ATEC sub-section score. All sub-section scores did not form a normal distribution so were analysed using the Related-samples Wilcoxon Signed Rank test.

The primary outcome was also tested using two different linear mixed models with percentage change in ATEC Total as the dependent variable in both models. Model 1 used fixed factors of time, treatment sequence, age at enrolment and treatment. Random factors were Participant ID nested within treatment sequence and the method was restricted maximum likelihood. Model 2 used fixed factors of time, treatment sequence, age at enrolment and treatment, with repeated measures of Time (Participant ID). The method was restricted maximum likelihood.

For each of the gastrointestinal symptoms on the Gastrointestinal History questionnaire, the null hypothesis is that there is no difference between Vivomixx and placebo in the change in frequency of symptoms from T0. The frequency assessments on the questionnaire were converted to a numeric value to facilitate analysis in the following way; Never=0, Rarely=1, Sometimes=2, Frequently=3 and

Always=4. All the Gastrointestinal History scores were analysed using the Related-samples Wilcoxon Signed Rank test as they did not conform to a normal distribution and also with Chi squared as it was considered that the data may not be entirely discrete.

For the Aberrant Behaviour Checklist (ABC), the null hypothesis for each of the section scores is that there is no difference between Vivomixx and Placebo in the change from T0 score. The scores for Hyperactivity/Non-compliance formed a normal distribution so this was assessed using a paired samples t-test. All other section scores did not form a normal distribution so were assessed using the Related-samples Wilcoxon signed rank test.

The null hypothesis for the Autism Parenting Stress Index (APSI) is that there is no difference between Vivomixx and placebo in the change from T0 score. The scores did not form a normal distribution, so the Related-samples Wilcoxon signed rank test was used.

The null hypothesis for the Bristol Stool Scale is that there would be no difference between Vivomixx and placebo in the absolute risk and relative risk of a Type 4 stool. These were calculated manually along with their corresponding 95% CI, according to published formulae. 95% confidence intervals that do not contain the value 1 are statistically significant at alpha = 0.05.

The null hypothesis for the Clinical Global Impression score for gastrointestinal symptoms and behaviour is that there was no difference between Vivomixx and placebo in the change from T0. These scores did not form a normal distribution, so they were analysed using the Related-samples Wilcoxon signed rank test.

The null hypothesis for the Researcher and Primary Care giver ratings of treatment effectiveness is that there was no difference between Vivomixx and placebo. These scores did not form a normal distribution, so they were analysed using the Related-samples Wilcoxon signed rank test.

3.4.4 Per protocol analysis

All outcome measures were tested for normal distribution using One-sample Kolmogorov-Smirnov test and Q-Q plots to determine whether to use non-parametric testing or paired T-test.

The per protocol (PP) group consisted of the ITT group less those participants that met the following criteria;

1. Had taken antibiotics during the study period, n=6

2. Had started taking a prescription medication that could affect the results of the ATEC questionnaire, n=1

3. Had consumed less than 75% of the recommended number of sachets of Vivomixx over the course of the study, n=2

The statistical analysis method was the same as for the ITT analysis except that the ABC Lethargy/social withdrawal scores and the ATEC Health, Physical, Behaviour both conformed to a normal distribution in the PP group, so they were analysed using a paired t-test.

3.4.5 Order of treatment analysis

The percentage change in ATEC Total from T0 after Vivomixx and after placebo for the two different sequences of treatment were analysed to see if the order of treatment was significant. The 2-samples independent T-test was used. A linear mixed model was also constructed to test the order-of-treatment effect where the percentage change in ATEC Total from T0 was the dependent variable and Time was a fixed effect, (Time=1 indicated after Part 1, and Time=2 indicated after Part 2). Sequence was the other fixed effect in the model (Sequence=0 indicated Placebo-Vivomixx and Sequence=1 indicated Vivomixx-placebo).

3.4.6 Post-hoc responder analysis

The results of the statistical analysis of the outcome measures was shared with the Data Monitoring Committee. It was noted that a number of participants (n=22) had a strong response to Vivomixx, as defined by a greater than 15% decrease in the ATEC Total score from T0. This definition was based on clinical experience and the definition

of significant improvement in other studies using ATEC Total as an outcome measure [218] On the direction of the Data Monitoring Committee, a post-hoc responder analysis was conducted with the guidance of the independent study statistician. As there were many variables that might contribute to a participant being a strong responder, a Principle Components Analysis (PCA) was used to find the most likely variables to focus on. Following this, Chi squared analysis was used to test whether there was a significant relationship between the Strong Responder marker and the categorical variables identified. Similarly, Spearman's rank correlation was used to assess the relationship between being a Strong Responder and T0 diarrhoea frequency on the Gastrointestinal History questionnaire.

3.5 Results

3.5.1 Characteristics of the participants

All 69 participants had a diagnosis of either autism or autism spectrum disorder. 56 of the diagnoses were made by an NHS multi-disciplinary team, 9 by an NHS consultant and 4 by other health professionals outside of the NHS. There were no statistically significant differences in the characteristics for the two order of treatment groups, as shown in

Table 3-1. The spread of ages within the two order-of-treatment groups was also not significantly different, Fisher-Freeman-Halton Exact test, 10.972, Exact significance .437.

Table 3-1; Participant characteristics

Participant characteristic	Total group	Vivomixx-Placebo	Placebo-Vivomixx	Sig (2-tailed)
Age at enrolment (years)				
Mean (SD)	7.8 (2.6)	8.3 (2.3)	7.3 (2.9)	0.109
Minimum	3	4	3	
Maximum	14	14	14	
Gender				
Female, count (%)	12 (17.4)	4 (11.4)	8 (23.5)	Chi squared (2 sided)
Male, count (%)	57 (82.6)	31 (88.6)	26 (76.5)	0.185
Ethnicity, count (%)				
Arab	2 (2.9)	1 (2.9)	1 (2.9)	
Asian/Asian British	6 (8.7)	3 (8.6)	3 (8.8)	
Black/African/Caribbean/ Black British	4 (5.8)	2 (5.7)	2 (5.9)	Fisher Exact (2 sided) .867
Chinese	1 (1.4)	0	1 (2.9)	
Hispanic	1 (1.4)	0	1 (2.9)	
Mixed/Multiple ethnic	12 (17.4)	8 (22.9)	4 (11.8)	
White	43 (62.3)	21 (60.0)	22 (64.7)	
T0 ATEC Total score				
Mean (SD)	73.2 (27.8)	70.2 (25.3)	76.3 (30.2)	0.364
Additional diagnoses				Chi squared (2 sided)
ADHD	11	7	4	.350
Learning disability	22	10	12	.549

3.5.2 Safety and adherence to protocol

There were no serious adverse events reported. The incidents of adverse events and protocol adherence events is listed in Table 3-2.

Table 3-2; Safety and adherence events

	Number while taking Vivomixx	Number at risk - Vivomixx	Number while taking placebo	Number at risk - placebo
Adverse event	10	65	16	68
Participant withdrawal	1	65	3	68
Participant lost to follow-up	3	65	1	68
Participant not reaching full dose	6	65	4	68

The results of the absolute risk and the relative risk of various events while taking Vivomixx compared to while taking placebo, is given in Table 3-3. The absolute risk of having an adverse event while taking Vivomixx is 0.08, which is 8% lower than while taking placebo, $p < 0.05$. The relative risk of having an adverse event with Vivomixx compared to placebo is 0.65, which means that the chance of having an adverse event with Vivomixx is 65% of the risk with placebo, although this is not statistically significant. The absolute risk of a participant withdrawing while taking Vivomixx is 0.029 which is 2.9% lower than with placebo $p < 0.05$. The relative risk of a participant withdrawal with Vivomixx compared to placebo is 0.349, which means that the risk of a withdrawal with Vivomixx is 35% of the risk with placebo, but this is not statistically significant. The absolute risk of a participant being lost-to-follow-up while taking Vivomixx is 0.032 which is 3.2% higher than with placebo $p < 0.05$. The relative risk of a participant being lost-to-follow-up with Vivomixx compared to placebo is 3.143, which means that the risk of a being lost-to-follow-up with Vivomixx is 3-fold higher than the risk with placebo, but this is not statistically significant. The absolute risk of a participant not reaching the full dose while taking Vivomixx is 0.034, which is 3.4% higher than with placebo $p < 0.05$. The relative risk of a participant not reaching the full dose with Vivomixx compared to placebo is 1.57, which means that the risk of not reaching the full dose with Vivomixx is 1.5-fold higher than the risk with placebo, but this is not statistically significant.

Table 3-3; Comparison of safety and adherence

	Proportion while taking Vivomixx	Proportion while taking placebo	Absolute risk while taking Vivomixx (95%CI)	P value	Relative risk while taking Vivomixx (95%CI)	P value
Adverse event	0.154	0.235	0.08 (-0.053 to 0.213)	<0.05	0.65 (0.321 to 1.334)	>0.05
Participant withdrawal	0.015	0.044	0.029 (-0.085 to 0.027)	<0.05	0.349 (0.037 to 3.264)	>0.05
Lost-to-follow-up	0.0462	0.0147	0.032 (-0.027 to 0.09)	<0.05	3.143 (0.335 to 29.4)	>0.05
Participant not reaching full dose	0.0923	0.0588	0.034 (-0.003 to 0.182)	<0.05	1.57 (0.464 to 5.3)	>0.05

3.5.3 Intention to treat results

3.5.3.1 Primary outcome measure ATEC Total percentage change from T0 Vivomixx vs placebo

There was no statistically significant difference between ATEC Total percentage change from T0 after Vivomixx, mean (M) -12.122, standard deviation (SD) 20.9 compared to after placebo, M -11.427, SD 20.3; t(63) -.276, p=0.784. In linear mixed Model 1, Vivomixx vs placebo was not significant in determining the percentage change in ATEC Total from T0 (F .162, numerator df 1, denominator df 62, p=0.689). In linear mixed Model 2, Vivomixx vs placebo was not significant in determining the percentage change in ATEC Total from T0 (F .162, numerator df 1, denominator df 62, p=0.689). All analyses support maintaining the null hypothesis for the primary outcome.

3.5.3.2 Secondary outcome measures

3.5.3.2.1 ATEC subsection scores

There was no statistically significant difference between the percentage change from T0 after Vivomixx compared to after placebo for any of the ATEC subsection scores. The results of the Related-samples Wilcoxon Signed Rank tests are shown in Table 3-4.

Table 3-4; ATEC subsections comparison of percentage change following Vivomixx vs placebo

ATEC subsection	Z	p
Speech, language, communication	-.283	0.777
Sociability	.392	0.695
Sensory Cognitive Awareness	-.023	0.982
Health, physical, behaviour	.891	0.373

3.5.3.2.2 Gastrointestinal History

These results were analysed in two different ways as there was some debate about whether the data was truly discrete. However, there were no statistically significant differences for any of the GIH scores between change from T0 after Vivomixx compared to after placebo (see

Table 3-5 and Table 3-6).

Table 3-5; GIH Chi squared results

GIH Section	df	N	X^2	p	Fisher exact	p
Abdominal pain difference	6	132	6.623	.342	6.145	.363
Gaseousness difference	7	132	4.597	.769	4.841	.726
Diarrhoea difference	6	132	5.179	.555	4.799	.597
Constipation difference	8	132	5.140	.844	5.101	.837
Pain on stooling difference	7	132	2.542	.944	3.122	.927
Vomiting difference	5	132	1.969	.906	2.249	.906

Table 3-6; GIH Related-samples Wilcoxon Signed Rank results

GIH section	Z	p	N
Abdominal pain difference	.920	0.357	66
Gaseousness difference	-1.059	0.290	66
Diarrhoea difference	.810	0.418	66
Constipation difference	-.340	0.734	66
Pain on stooling difference	.912	0.362	66
Vomiting difference	.378	0.705	66

3.5.3.2.3 Bristol Stool Scale

The following charts illustrate the proportion of each stool type at T0 (Figure 3-3), after taking Vivomixx (Figure 3-4) and after taking placebo (Figure 3-5).

Figure 3-3; Bristol stool type at T0 (valid percentage)
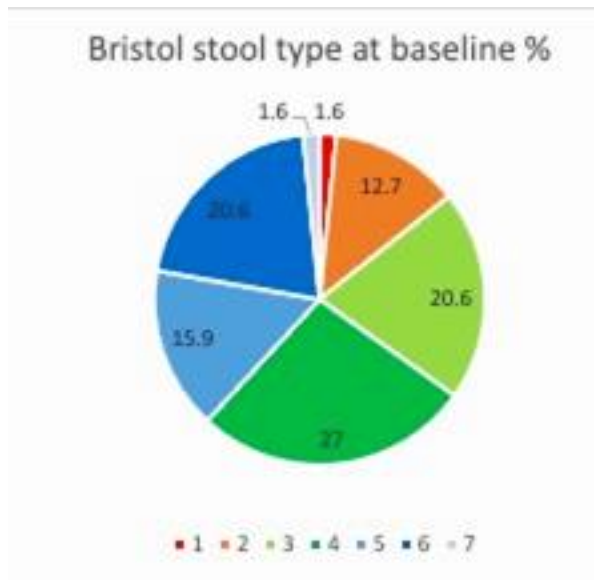
Figure 3-4; Bristol stool type after Vivomixx (valid percentage)
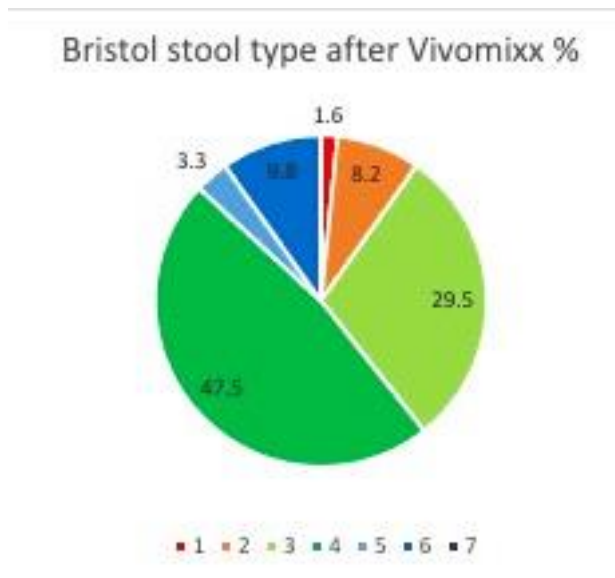
Figure 3-5; Bristol stool type after placebo (valid percentage)

Bristol stool type after placebo %
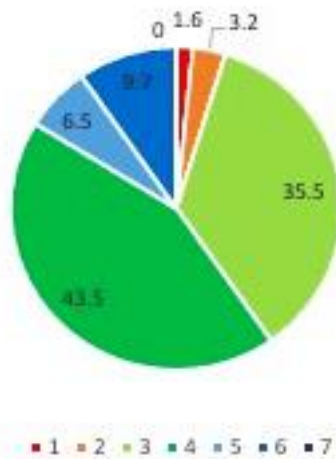
Table 3-7; Frequency of Type 4 stools after Vivomixx and after placebo

	Type 4 stool	NOT Type 4 stool	Total
Vivomixx	29 (47.5%)	32 (52.5%)	61
Placebo	27 (43.5%)	35 (56.5%)	62
Total	56 (45.5%)	67 (54.5%)	123

The proportion of participants having Type 4 stools after taking Vivomixx is 0.475.

The proportion of participants having Type 4 stools after taking placebo is 0.435.

The absolute risk (chance) of having Type 4 stools after taking Vivomixx is 0.04, 4% higher than the after placebo, 95% CI = -0.14 to 0.22, p <0.05.

Relative risk (chance) of having a Type 4 stool after Vivomixx compared to placebo is 1.09. This means that the chance of having Type 4 stool after Vivomixx is 109% of the chance after placebo, but this is not statistically significant, 95% CI = 0.74 to 1.61, p>0.05.

3.5.3.2.4 Aberrant Behaviour Checklist

There were no statistically significant differences between the change from T0 after Vivomixx compared to after placebo for all the section scores. The results for the sections scores analysed non-parametrically are given in Table 3-8. There was no statistically significant difference between ABC Hyperactivity/Non-compliance change from T0 after Vivomixx (M -2.42, SD 7.434) compared to after placebo (M -2.16, SD 7.037); t (61) = -.248, p = 0.805.

Table 3-8; ABC Related-samples Wilcoxon Signed Rank results

ABC section	Z	p	N
Irritability, agitation and crying	.475	0.635	62
Lethargy/social withdrawal	-.902	0.367	62
Stereotypic behaviour	.511	0.609	62
Inappropriate speech	.018	0.985	62

3.5.3.2.5 Autism Parenting Stress Index

There was no significant difference between Vivomixx and placebo in the change from T0 of the Autism Parenting Stress Index : Z = .439, p=0.661 (2-sided test). This is helpful as it indicates that giving Vivomixx caused no more stress to the parents than giving the placebo.

3.5.3.2.6 Clinical Global Impression

There was no significant difference between Vivomixx and placebo in the change from T0 of the Clinical Global Impression for gastrointestinal symptoms : Z = .534, p=0.593 (2-sided test). There was also no significant difference between Vivomixx and placebo in the change from T0 of the Clinical Global Impression for behaviour : Z = .721, p=0.471 (2-sided test).

3.5.3.2.7 Researcher and Primary Carer rating of treatment effectiveness

There was no significant difference between Vivomixx and placebo in the Researcher rating of treatment effectiveness: Z = -.745, p=0.457 (2-sided test). There was also no significant difference between Vivomixx and placebo in the Primary care giver rating of treatment effectiveness: Z = .314, p=0.753 (2-sided test).

3.5.4 Per protocol analysis

3.5.4.1 Primary outcome measure ATEC Total percentage change from T0 Vivomixx vs placebo

There was no statistically significant difference between ATEC Total percentage change from T0 after Vivomixx (M -12.629, SD 21.1) compared to after placebo (M -11.985, SD 18.6); t (49) = -.240, p=0.811.

3.5.4.2 Secondary outcome measures

3.5.4.2.1 ATEC subsection scores

There was no statistically significant difference between the percentage change from T0 after Vivomixx compared to after placebo for any of the ATEC subsection scores analysed using non-parametric testing (see Table 3-9). There was no statistically significant difference between ATEC Health, Physical, Behaviour percentage change from T0 after Vivomixx (M -18.181, SD 25.4) compared to after placebo (M -12.779, SD 25.2);   t (49) = -1.354, p=0.182.

Table 3-9; ATEC subsections Related-samples Wilcoxon signed rank

ATEC subsection	Z	p
Speech, language, communication	-.314	0.753
Sociability	.373	0.709
Sensory Cognitive Awareness	-.502	0.615

3.5.4.2.2 Gastrointestinal History

These results were analysed in two different ways as there was some debate about whether the data was truly discrete. However there were no statistically significant differences for any of the GIH scores between change from T0 after Vivomixx compared to after placebo (see Table 3-10 and

Table 3-11).

Table 3-10; GIH Chi squared results

GIH Section	df	N	X^2	p	Fisher exact	p
Abdominal pain difference	6	114	6.473	.361	5.985	.389
Gaseousness difference	7	114	5.580	.637	5.856	.573
Diarrhoea difference	6	114	5.065	.573	4.721	.605
Constipation difference	8	114	5.606	.778	5.598	.765
Pain on stooling difference	6	114	1.973	.962	2.223	.965
Vomiting difference	4	114	1.245	.873	1.574	.873

Table 3-11; GIH Related-samples Wilcoxon signed rank results

| GIH section	Z	p	N
Abdominal pain difference	-1.340	0.180	56
Gaseousness difference	.730	0.290	56
Diarrhoea difference	-.233	0.816	56
Constipation difference	.285	0.775	56
Pain on stooling difference	-.552	0.581	56
Difficulty swallowing difference	-.447	0.655	56

3.5.4.2.3 Bristol Stool Scale

The following charts illustrate the proportion of each stool type at T0 (Figure 3-6), after taking Vivomixx (Figure 3-7) and after taking placebo (Figure 3-8).

**Figure 3-6; Bristol stool type at T0 (valid percentage)**
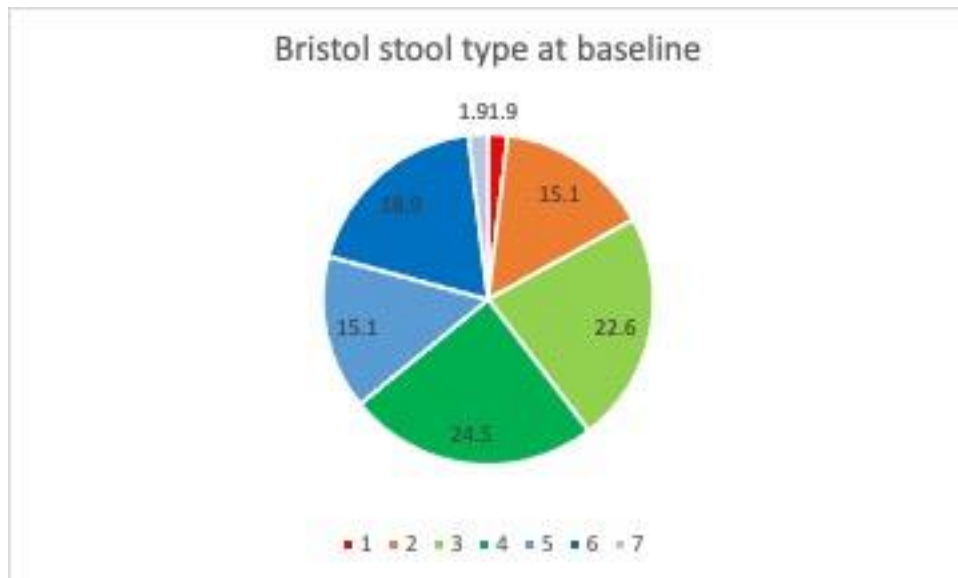
Figure 3-7; Bristol stool type after Vivomixx (valid percentage)

Bristol stool type after Vivomixx
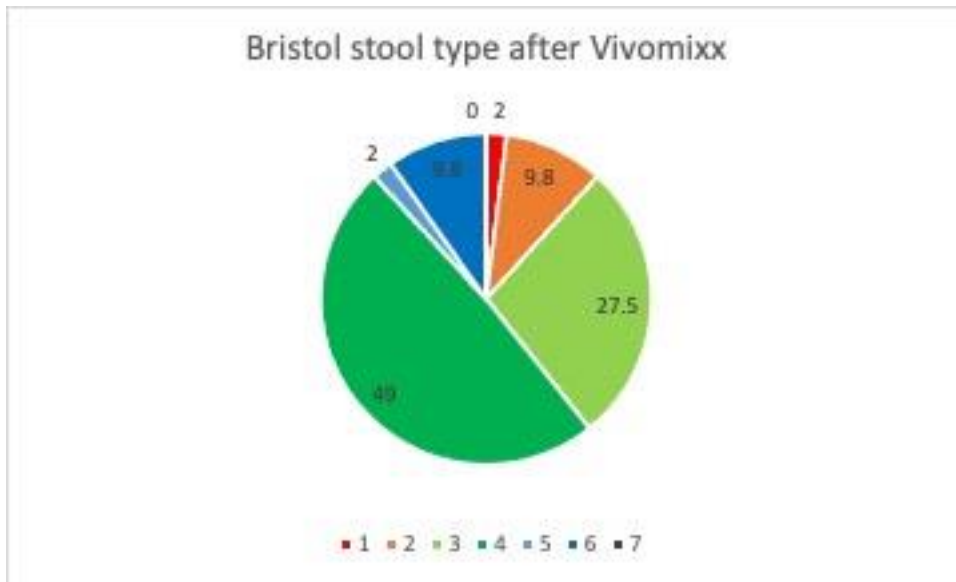
Figure 3-8; Bristol stool type after placebo (valid percentage)

Bristol stool type after placebo
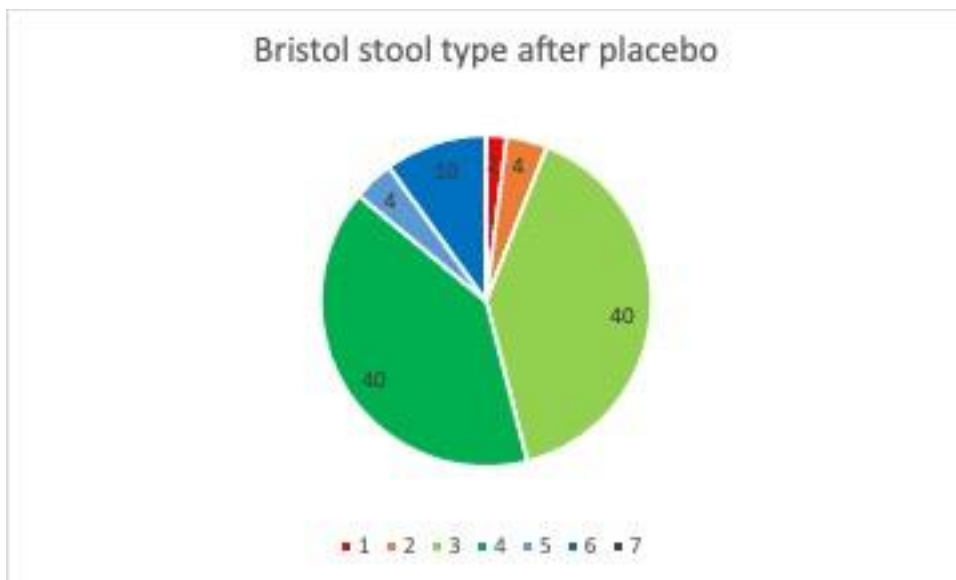
Table 3-12; Frequency of Type 4 stools after Vivomixx and after placebo

	Type 4 stool	NOT Type 4 stool	Total
Vivomixx	25 (49.0%)	26 (51.0%)	51
Placebo	20 (40.0%)	30 (60.0%)	50
Total	45 (44.6%)	56 (55.4%)	101

The proportion of participants having Type 4 stools after taking Vivomixx is 25/51 = 0.49.

The proportion of participants having Type 4 stools after taking placebo is 20/50 = 0.40.

The absolute risk (chance) of having Type 4 stools after taking Vivomixx is 0.09, 9% higher than the after placebo, 95% CI -0.10 to 0.28, p <0.05.

Relative risk (chance) of having a Type 4 stool after Vivomixx compared to placebo is 0.49/0.40 = 1.225. This means that the chance of having Type 4 stool after Vivomixx is 122.5% of the chance after placebo, although this is not statistically significant, 95% CI 0.79 to 1.902, p>0.05.

3.5.4.2.4 Aberrant Behaviour Checklist

There were no statistically significant differences between the change after Vivomixx from T0 compared to after placebo for all the section scores analysed non-parametrically (see Table 3-13). There was no statistically significant difference between ABC Hyperactivity/Non-compliance change from T0 after Vivomixx (M -3.24, SD 7.204) compared to after placebo (M -2.88, SD 6.595); t (49) = -.310, p=0.758. There was no statistically significant difference between ABC Lethargy/Social Withdrawal change after Vivomixx from T0 (M -2.76, SD 7.150) compared to after placebo (M -3.70, SD 6.335); t (49) = .938, p=0.353.

Table 3-13; ABC Related-samples Wilcoxon signed rank results

ABC section	Z	p	N
Irritability, agitation and crying	.882	0.378	50
Stereotypic behaviour difference	.526	0.599	50
Inappropriate speech difference	-.455	0. 649	50

3.5.4.2.5 Autism Parenting Stress Index

There was no significant difference between Vivomixx and placebo in the change from T0 in the Autism Parenting Stress Index : Z = -.776, p=0.438 (2-sided test). This is helpful as it indicates that giving Vivomixx caused no more stress to the parents than giving the placebo.

3.5.4.2.6 Clinical Global Impression

There was no significant difference between Vivomixx and placebo in the change from T0 for the Clinical Global Impression score for gastrointestinal symptoms : Z = .704, p=0.482 (2-sided test). There was also no significant difference between Vivomixx and placebo in the change from T0 for the Clinical Global Impression score for behaviour : Z = 1.417, p=0.156 (2-sided test).

3.5.4.2.7 Researcher and Primary Carer rating of treatment effectiveness

There was no significant difference between Vivomixx and placebo in the Researcher rating of treatment effectiveness: Z = .901, p=0.367 (2-sided test). There was also no significant difference between Vivomixx and placebo in the Primary care giver rating of treatment effectiveness: Z = -.584, p=0.559 (2-sided test).

3.5.5 Order of treatment analysis

The descriptive statistics for the percentage change in ATEC Total for each of the treatment order groups and for each of the treatments is shown in Table 3-14.

Table 3-14; Order of treatment effect using the primary outcome

Treatment order	Treatment	N	Mean % change ATEC Total	Std. Dev.	Std. Error Mean
Placebo-Vivomixx	Vivomixx	31	-16.1	20.34	3.65
Vivomixx-placebo	Vivomixx	33	-8.3	21.04	3.66
Placebo-Vivomixx	Placebo	31	-10.8	17.25	3.10
Vivomixx-placebo	Placebo	33	-12.0	23.07	4.02

There was not a significant difference in the percentage change of ATEC Total after Vivomixx in the Placebo-Vivomixx group (M -16.1 , SD 20.34) compared to the Vivomixx-placebo group (M -8.3 , SD 21.04); t(62) = 1.51 , p=0.137. There was not a significant difference in the percentage change of ATEC Total after Placebo in the Placebo-Vivomixx group (M -10.8, SD 17.25) compared to the Vivomixx-placebo group (M -12.0, SD 23.07); t(62) = 0.23 , p=0.816.

A linear mixed model was also used to test the order of treatment effect using the percentage change in ATEC Total from T0 as the dependent variable and using Time as a fixed effect where, after Part 1, Time=1 and after Part 2, Time=2. Sequence was the other fixed effect in the model and Sequence=0 indicated Placebo-Vivomixx and Sequence=1 indicated Vivomixx-placebo.   Neither Time nor Sequence were significant in predicting the percentage change in ATEC Total from baseline indicating there was no significant order-of-treatment effect.

3.5.6 Responder analysis

There were 22 participants who met the criteria for strong response to Vivomixx as defined by a 15% or more reduction in ATEC Total from baseline. The characteristics of these Strong Responder participants are compared to participants without a strong response and also to all participants in Table 3-15.

Table 3-15; Participant characteristics comparison

Participant characteristic	Strong responders	Other response	Total group
Age at enrolment (years)			
Mean (SD)	7.5 (2.7)	8.0 (2.6)	7.8 (2.6)
Minimum	4		3
Maximum	14		14
Gender			
Female, count (%)	4 (18.2)	8 (17.0)	12 (17.4)
Male, count (%)	18 (81.8)	39 (83.0)	57 (82.6)
Ethnicity, count (%)			
Arab	0	2 (4.3)	2 (2.9)
Asian/Asian British	1 (4.5)	5 (10.6)	6 (8.7)
Black/African/Caribbean/Black British	2 (9.1)	2 (4.3)	4 (5.8)
Chinese	1 (4.5)	0	1 (1.4)
Hispanic	1 (4.5)	0	1 (1.4)
Mixed/Multiple ethnic	2 (9.1)	10 (21.3)	12 (17.4)
White	15 (68.2)	28 (59.6)	43 (62.3)
Baseline ATEC Total score			
Mean (SD)	76.7 (29.6)	71.6 (27.1)	73.2 (27.8)
GIH T0 scores			
Bristol stool type, mean (median)	4.3 (4)	4.0 (4)	
Abdominal pain, mean (median)	1.5 (1.5)	2.0 (2)	
Gaseousness, mean (median)	2.3 (2)	2.5 (3)	
Diarrhoea, mean (median)	2.1 (2)	1.6 (1.5)	
Constipation, mean (median)	2.1 (2)	2.0 (2)	
Pain on stooling, mean (median)	1.7 (2)	1.7 (2)	
Vomiting, mean (median)	0.4 (0)	0.4 (0)	
Difficulty swallowing, mean (median)	0.1 (0)	0.3 (0)	

Following the Principle Components Analysis, three variables were identified as candidates to assess for correlation with the strong responder marker: These were low fibre diet indicator; food supplement user; and T0 diarrhoea frequency.

Neither Low Fibre Diet nor Food Supplement User were found to have a significant association with the Strong Responder marker using Pearson Chi-Square test:

Low Fibre Diet X^2 (1, N=55) = 2.30, p= .129

Food Supplement User X^2 (1, N=55) = 1.75, p= .186

Spearman's rank correlation was used to assess the relationship between T0 diarrhoea frequency and Strong Responder status. There was a positive correlation between the two variables, R(53) = .27, p= .051. This relationship was further investigated by plotting a clustered bar chart Figure 3-9. From the chart it appears that those participants who reported not suffering any diarrhoea at T0, were less likely to have a strong positive response to Vivomixx.

Figure 3-9; Strong response vs other response for T0 diarrhoea frequency

113
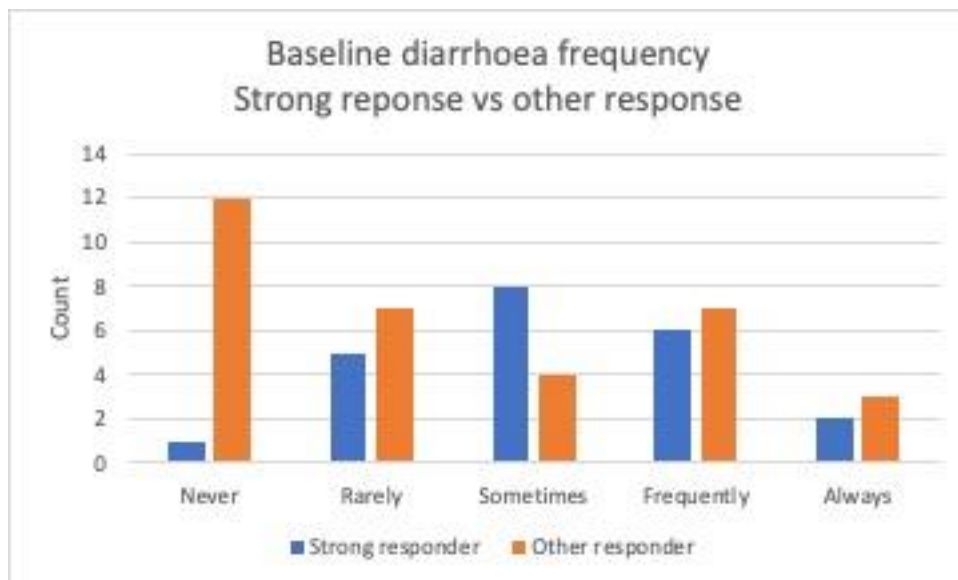
3.6 Limitations and strengths of the study

The study protocol included stool microbiome analysis for a small number of participants at three timepoints (enrolment, after Vivomixx and after placebo) but Covid-19 lockdown interrupted the collection and storage of stool samples which meant that insufficient samples were collected for any meaningful analysis. Therefore, all outcome measures were parent reported assessments. Although parents have been shown to be comparable with health professionals when reporting their child's global function and GI symptoms [197] [202] [148], they are known to report a strong placebo effect [207] and this can make it difficult to determine a real treatment effect. To mitigate this, the child's educator was asked to complete ATEC and ABC questionnaires at the three time points, since educators and health professionals do not demonstrate the same placebo effect as parents [219]. Unfortunately, there was a very poor response rate and insufficient data to complete any analysis. The validity of the Educator questionnaire data was also affected by different educators completing a child's questionnaire due to various factors (e.g. staff changes, the child moving class or the child changing school). Finally school closures during Covid-19 lockdown impacted our ability to get the Educator Questionnaires completed and returned. This is a real-world study conducted during a challenging time and suffered from the difficulties that entails.

The ATEC was chosen as the primary outcome measure due to its demonstrated sensitivity to change. Despite this, it may not be sensitive enough to detect a treatment effect in those autistic children with a low baseline score. Some autistic children display significant day-to-day variability in global functioning and although the primary carers were instructed to consider the previous two weeks in completing all the assessments, this may still have played a part in clouding the data.

Strengths of this study were its double-blind placebo-controlled crossover design. The crossover study directly compares whether a participant is better on Vivomixx or placebo. This differs from studies with a treatment group and a control group, even when matched by age, gender and impact of autism (e.g. ADOS score), due to the wide heterogeneity within the autism diagnosis. The draft protocol and study documents as well as the proposed outcome measures were reviewed by members of the autism community. The conduct of the study was guided by an independent

trial steering group which included members of the autism community and also by a data monitoring committee. Another strength of this study was that recruitment was open to all autistic children with persistent GI symptoms including those with minimal language and co-occurring learning disability. The sample size is large for a study of this kind and the drop-out rate was low (13%) which may reflect the fact that the study was designed to make continued involvement as easy as possible. The adherence rate in this study was unusually high which may reflect the Taste Test week and the 1:1 contact with the researcher every 4 weeks. The Taste Test week gave the child agency in the decision to participate and meant participants were effectively selected to tolerate the product.

3.7 Discussion

This was a robustly designed, and to my knowledge, the largest study published using a probiotic for autistic children. Although no difference was found between the response to Vivomixx compared to placebo in the group as a whole, it is interesting that a subgroup had a notable response to Vivomixx in terms of improvement in global function.

There are a number of possible reasons why this is a negative study. Although diet is known to be a strong influencer of the gut microbiome and so could be a confounding factor, many autistic children habitually choose to eat the same foods [220]. From the compliance data collected in the VIVO-ASD study, only one child out of 69 made a notable change in their diet during the 6-month participation in the study. At the time of the study design, there was no evidence to suggest that the type of GI symptom suffered by participants should be restricted. Since then, probiotic research has found that specific health issues respond to specific probiotic species and even specific strains within a species [221]. Given this new knowledge, there is a possibility that restricting enrolment to those with a single specific GI condition, may have shown a significant result. Set against this, recruitment to the study was difficult and time-consuming and further restriction of inclusion criteria would have increased this difficulty. The probiotic used in the VIVO-ASD study was a multi-species probiotic (Vivomixx, also known as VISBIOME) with proven efficacy for IBS in children. Given the more recent evidence on specificity of probiotics for health conditions, it should

perhaps be expected that different probiotics could show different results in this patient group. One other study [171] and one pilot study [173] used the same probiotic. The pilot study had a crossover design and participants were autistic children with GI symptoms and anxiety. They reported an improvement in the quality-of-life score and an improvement in GI symptoms on the probiotic but as expected for a pilot study, did not find any statistically significant results. The Santocchi study [171] used a crossover design and did not find any statistically significant results at the whole group level. A post-hoc analysis split the participants into those with GI symptoms (AUT-gastrointestinal) and those without GI symptoms (AUT-NoGI). For true comparison with the VIVO-ASD study, we should focus on their results in the AUT-GI group. Although they reported statistically significant improvements in GI symptoms, adaptive function and sensory processing, the dropout rate in this subgroup was 50% so results may be subject to attrition bias. Also, the numbers completing the study in this subgroup were small (9 who had the probiotic and 8 who had the placebo) and therefore statistical results may be swayed by outlier data.

The study populations of the autism probiotic studies (excluding the pilot studies and the case report) are not dissimilar to that of the VIVO-ASD study. The VIVO-ASD participants ranged in age from 3 years to 14 years with a mean age of 7.8 years. In comparison, the mean age of participants in the other autism probiotic studies in Table **1-1**, range from 9.2 years [222] to 4.2 years [171]. This is not dissimilar to the VIVO-ASD population, particularly when you exclude the Santocchi study [171] which is usual in the very young age of participants. The Santocchi study included participants from 18 months to 6 years old and since the gut microbiome is rapidly changing and developing up to the age of 3 years, this study population would be considered distinct to all the other autism probiotic studies including the VIVO-ASD study. There were 82.6% boys and 17.4% girls in the VIVO-ASD study, which is not dissimilar to the other studies (excluding pilot studies) where the percentage of boys ranges from 63.3% [223] to 91% [222][180].

The autism probiotic studies have taken place in six different countries with only Parracho et al being conducted in the UK. Given the latest findings on differing gut microbiota patterns in two different states in the USA [141], the geographical disparity may be a factor in the differing results. The Parracho study is geographically

comparable to the VIVO-ASD study and found an improvement in stool consistency compared to placebo, but no difference between the probiotic and placebo in behaviour assessment.   This study was as robustly designed as the VIVO-ASD study (double-blind, placebo-controlled crossover), importantly employing the most accurate control group for this very heterogeneous population.   However, as discussed in section 1.9 the Parracho study suffered attrition bias which may overstate results. The results of the VIVO-ASD study agree with the Parracho study in not finding a significant difference in behaviour assessment, but possibly contradict the stool consistency findings as no significant difference was found in the frequency of diarrhoea and constipation, although different measures were used.

In conclusion, this study has added a robustly designed study to the evidence base regarding the use of probiotics for autistic children with GI symptoms and confirmed the safety of Vivomixx. As a randomised double-blind placebo-controlled trial, a significant treatment effect was not found in the group as a whole, which is contrary to similar published open studies and underlines the importance of robust study design. Our study group was very mixed and included many participants with additional challenges such as learning disability and being non-verbal. Despite this, we were able to achieve good adherence for the group as a whole. This demonstrates the feasibility of intervention studies which include often overlooked groups within the autism diagnosis and should encourage researchers to include autistic children with extra challenges. Considering the results of this study and other autism probiotic studies, it is too early to dismiss probiotics as not effective for autistic children. A subgroup in this study experienced a notable improvement in global function after Vivomixx treatment suggesting that a more tightly defined subgroup may show positive results, and this warrants further research.

From a clinical perspective, our results suggest;

- For an autistic child who has some degree of diarrhoea (including intermittent or in combination with another GI symptom), they may benefit from a 3-month course of Vivomixx at the doses used in this study.

- For an autistic child without any diarrhoea but suffering from other bothersome GI symptoms, a trial of a different probiotic may be preferable for relieve of GI symptoms.

- In both cases, parents should monitor bowel movement frequency and consistency, as well as 2-3 other bothersome symptoms chosen by the child and parent, and review the effectiveness after the treatment course.

Chapter 4   In-depth Interviews

Recruitment to the VIVO-ASD study was more difficult than expected so I wanted to understand the experience of participants, their motivation for participating the in the study, the obstacles they encountered, the things that went well and their thoughts about how similar research might be designed in the future to make it more accessible to them. To achieve this, I conducted in-depth semi-structured interviews with 12 parents of participants from the VIVO-ASD study. To put their experiences into context I also asked them about the impact of their child's GI symptoms on their child and their family, day-to-day management of their child's GI symptoms and their experience of healthcare for their child's GI symptoms.

We sought to answer the following questions;

"What are the lived experiences of families managing persistent GI symptoms in their autistic child and what has been their experience of related healthcare?"

"What can we learn from the experience of participants in the VIVO-ASD clinical trial that could inform the design of future clinical trials to make them more accessible and acceptable to families with an autistic child with GI symptoms?"

We did not initially seek to answer a question about the effect of Covid-19 lockdown on autistic children's GI symptoms, but the interviews were done during the lockdown and the topic arose in the first few interviews. The topic guide was then altered to explore this subject leading to a third research question;

"How has Covid-19 lockdown and Covid-safe measures in schools affected the child's GI symptoms and the family experience of managing these symptoms?"

4.1   Introduction

There are currently no guidelines from professional gastroenterology bodies in the UK or USA for the assessment and treatment of GI distress specifically in autistic children. Consequently, GI symptoms in autistic children should receive the same standard treatment as non-autistic children. Therefore, to understand the analysis of the interview data, it is helpful to compare the experience with that of non-autistic (Non-A)

children. In Non-A children who develop constipation this typically happens between ages 2 - 4 years and they will typically recover within 6-12 months of therapy. However, in 30% of cases it will continue into puberty. Compare this to studies of autistic children where the majority of children are still suffering GI symptoms after one (86.7%) [57] or two years (73.2%) [224].

In Non-A children, the risk factors for developing constipation include psychological stress including bullying at school. Symptoms associated with childhood constipation include nausea, loss of appetite, weight loss, abdominal pain, vomiting and urinary incontinence. Painful defecation can lead to the child withholding stools and this can lead to an accumulation of faecal matter in the rectum and eventually to mega rectum and loss of rectal sensation [225]. Standard treatment of functional constipation should include education of the patient and parent, toilet training, laxatives for resolving impaction and maintenance of regular bowel movements, plus regular follow-up. Patient education can alleviate anxiety and increases involvement of the patient in self-management of the symptoms [225].

In Non-A children, if functional constipation is not treated effectively, faecal incontinence can develop over time. Faecal incontinence without the child realising can be a sign of more severe constipation. Faecal incontinence affects the child's quality of life more as they reach the teenage years [226]. For children that do not respond to standard treatment for constipation and faecal incontinence, it is difficult for the clinician to manage the condition as there is a lack of research evidence, and a complex interplay of physiological and emotional factors at play. Chronic constipation can drive behaviour and vice versa [64].

Associated health conditions are common and varied in autistic children [47] and amongst them GI symptoms often co-occur with other conditions of increased prevalence, including epilepsy, anxiety, intellectual disability and sleep disorders. Symptoms of co-occurring disorders and prescribed medication side effects can become interwoven into a complex medical picture. Added to this, there is evidence that autistic individuals face barriers in accessing healthcare on several fronts (e.g., challenges that stem from autistic characteristics, challenges at the point of clinical care, and other access barriers) [227].

4.2 Methodology

4.2.1 Participant selection

I conducted semi-structured interviews with parents of 12 autistic children with persistent GI symptoms. All participants in this study were parents of children who had previously participated in a clinical trial of Vivomixx probiotic (VIVO-ASD [217]). This larger study was approved by the National Health Service Health Research Authority and the study protocol and results have already been reported [217]. The participants were selected to achieve a range of experience from those participating in the VIVO-ASD study, such that we used the following criteria in making selection decisions for this qualitative study: two girls on the child dose; two boys on the child dose; two girls on the 11+ dose; two boys on the 11+ dose; two girls who withdrew before the end of the study; two boys who withdrew before the end of the study. Within these criteria, participants were invited to participate in reverse chronological order of completing the VIVO-ASD study as their experience was more recent. Where we were unable to fulfil one of the criteria, we invited participants in simple reverse chronological order of their completion date for the VIVO-ASD study. Invitations to participate and information on the topics that would be discussed were sent by email. We obtained informed consent from parents by email, and this was confirmed at the beginning of the videocall interview. 12 out of 13 parents that responded to the invitation agreed to take part. The parent that declined to take part felt they did not have enough to say as their child withdrew from the VIVO-ASD study after 4 weeks. Interviews were organized at the convenience of the parents and as such two of the interviews were done with both parents, nine were done with the mother only and one with the father only. We interviewed the parents of 3 girls on the child dose, 5 boys on the child dose, 1 girl on the 11+ dose, 2 boys on the 11+ dose and 1 boy that withdrew early from the VIVO-ASD study.

4.2.2 Characteristics of the participants

The interviewees were all parents of children who participated in the VIVO-ASD probiotic clinical trial at University College Hospital London. At the time of the interviews, the children were aged between 5 and 15 years old. (Table 4-1).

Table 4-1; Participants ages

Age at time of interview (yrs)	Number of participants
5	2
7	2
9	2
10	1
11	1
12	2
14	1
15	1

 All children had a diagnosis of autism, confirmed by letter from the team who gave the diagnosis. The children had a variety of GI diagnoses (Table 4-2) and a mixture of GI symptoms (Table 4-3).   Participants were geographically spread across England. The participants included children identified by their parents as Asian/Asian British, Mixed/Multiple ethnic and White. Specific data on socioeconomic status was not recorded in the study. No incentive was offered to participate.

Table 4-2; Participants gastrointestinal diagnoses

Gastrointestinal diagnosis (current or previous)	Number of participants
Functional constipation	2
Reflux	3
Coeliac disease	1
Impacted bowel	1
Immature bowel	1
Rumination syndrome	1

Helicobacter pylori infection	1
Duodenal ulcer	1
Mega bowel	1
Irritable bladder	1
Enuresis	1
No gastrointestinal diagnosis	6

Table 4-3; Participants current gastrointestinal symptoms

Current gastrointestinal symptoms	Interviewee
Constipation managed with 3 sachets of Movicol daily but with regular soiling of underwear.	1
Odorous flatulence with slightly loose, sticky stools	2
Impacted, immature bowel, celiac, food allergies, swinging between severe constipation and diarrhoea, with some faecal incontinence at night	3
Vomiting after meals, and not yet toilet trained	4
Occasional stomach-ache, not affecting appetite, otherwise no symptoms	5
Constipation managed with ½ sachet of Movicol and several lifestyle measures, but needs very careful attention by parents	6
Bloating and odorous flatulence	7
Occasional undigested food in stools, otherwise no symptoms	8
Constipation, irritable bladder, nocturnal enuresis, urgency for bowel movements, occasional daytime soiling.	9
No current symptoms	10
Constipation with irregular bowel movements, not toilet trained for stools	11
Occasional very large stools, otherwise no symptoms	12

4.2.2.1 Habitual diets

Picky eating and aversion to vegetables and new foods in autistic children has been well researched [228] and is not the focus of this research. Research is conflicted on whether habitual diets in autistic children drives gastrointestinal symptoms or not [149][229]. However, two different stool microbiome patterns have been found in autistic children following diets either low or high, in legumes, fruit, vegetables, nuts and seeds. Thus, a brief description of the diets of participants is given here. Half of the participants had an intake of fruit and vegetables that was limited in quantity and also variety. The other half had a good intake and variety of fruit and vegetables in their diet. One child was vegetarian, and another did not eat meat but ate other sources of animal protein. One parent stated that their child didn't drink enough, and three parents felt their child's diet would benefit from more fibre. All other participants felt their child had sufficient fibre in their diet and sufficient hydration.

All the parents were aware of the importance of fruit and vegetables and sufficient hydration in their child's health, and they worked hard to encourage intake of these. There was less awareness of the need to include wholegrains and legumes in their child's diet for their contribution to dietary fibre. Only one parent mentioned that their child ate nuts, and none mentioned seeds. Overall, there was a lot of variety between the habitual diets of the children.

4.2.2.2 Behaviour around food

Five parents stated that their child was not a fussy eater. The other seven children were rigid about food choices to various degrees: This sometimes meant the child would only eat two different meals for dinner and food sometimes had to be specific brands. A sensitivity to food texture was common and some children also had a sensitivity to smell and taste. Most children enjoyed their food, and some were very motivated by food. New foods were challenging for many of the children and for some children, new foods could be a source of anxiety. Overall, there was significant variety in the behaviour of the children regarding food.

4.2.3 Researcher Characteristics

The analysis of the interviews was done in conjunction with another independent researcher to improve the rigour of the research. Both researchers were based at the GI physiology department in University College Hospital London, one as a specialist bowel nurse and the other as a graduate researcher studying dietary approaches to managing GI symptoms in autistic children. One researcher met with all the participants as part of the VIVO-ASD study, and the other researcher had no contact with participants. One researcher has personal family experience of autism.

4.2.4 Semi-structured interviews

A topic guide for the interviews was developed and then reviewed by an experienced public health qualitative researcher. Following the review, a number of revisions were made to the topic guide. It was then reviewed by three parents with their autistic child (where the child was able to participate) for comments on relevance, appropriate wording and any needed additions. The topic guide was used as a basis for the interviews. I conducted the interviews by videocall between 25th August 2020 and 18th January 2021. I made situational notes and reflections about the interview at the time of the interview which were considered in the analysis. The interviews lasted 60 minutes on average (range: 41 – 90 minutes) and were audio-recorded and transcribed verbatim except for anonymising any personal details. The transcript was shared with the interviewee and confirmation sought that it was a true reflection of the conversation.

4.2.5 Data Analysis

We used Framework analysis of these data which is suited to the research focus on experiential data and automatically builds an audit trail to improve the robustness of the research [230]. Framework analysis is not bound by a particular theoretical position, is flexible and can be shaped by the data. This makes it a better fit than Thematic analysis as there were certain subjects I wanted to explore but also wanted to be open to other subjects emerging from the data.

We familiarised ourselves with the data by reading all the transcripts and discussing them together. Following this a list of framework categories was developed which were primarily based on the subjects in the topic guide. This initial framework was

125

piloted on five transcripts by each of us independently to check whether it was a good fit for the data. Once the Framework was agreed, my colleague and I indexed all the transcripts independently and held regular meetings to discuss the emerging themes and the fit of the Framework. The indexed data were summarised and charted independently by my colleague and I into an Excel spreadsheet, along with selected quotes from interviewees that illustrated topical themes. Each category for each participant was analysed to identify recurring themes and inter-relationships and to identify a structure from the coded interview content, referring back to the interview transcripts where necessary. The result was written as a narrative which was discussed by the research team and then presented to the GI physiology team at University College Hospital London. Discussing the results with the clinical team helped to sense-check the findings and further shape the mapping and interpretation.

4.3 Results of Framework analysis

4.3.1 Family experience of managing GI symptoms in their autistic child and related healthcare

In answer to the question "What are the lived experiences of families managing persistent GI symptoms in their autistic child and what has been their experience of related healthcare?" our analysis revealed four major themes and one sub-theme;

- Gastrointestinal symptoms impact on many aspects of the lives of autistic children and their families and the impact tends to increase with age.
    - Sub-theme – Managing GI symptoms in autistic children is a source of stress for parents.
- Understanding the nature and severity of GI symptoms in autistic children is complex and multifactorial.
- Access to healthcare services for autistic children with GI symptoms is variable and often limited, with diagnostic overshadowing.
- Reasonable adjustments to the current NHS service are needed to reduce child and parent stress.

4.3.1.1 THEME 1 – Gastrointestinal symptoms impact on many aspects of the lives of autistic children and their families and the impact tends to increase with age.

4.3.1.1.1 Protracted experience of GI symptoms

Most of the children had been suffering from GI symptoms for a protracted period of time ranging from 2 years to 15 years. The children with constipation and hard stools that were painful to pass, would start withholding and would not open their bowels for 2-4 days or longer and sometimes would only pass stool in their sleep. Children often suffered some degree of faecal incontinence during the day and at night. When passed, the stools were very large leading to tummy aches and anxiety about going to the toilet. This pattern of symptoms was reported to be suffered for extended periods of time, 2 - 5 years. Protracted experience of other GI symptoms was also reported including reflux for 8 years that worsened over time to 2 hours of vomiting after every meal, and night-time enuresis and constipation managed by Movicol for 15 years.

4.3.1.1.2 Impact on the child of GI symptoms

Quality of life effects

Unpredictable GI symptoms were limiting the things some of the children felt comfortable doing;

> ".. he often had some faeces in his pants during the day and stuff and like that, which made it very uncomfortable for him. It meant that he didn't want to take baths, because he was worried that some would come out in the bath or go swimming. Things like that. So it really stood in the way of his quality of life, actually, looking back." [Interviewee 10]

> "It affects her life, if she wants to go and stay with friends and things like that. At her age, it's a bit embarrassing." [Interviewee 9]

Constipation had a major impact on the quality of some children's lives as it affected their ability to be independent in toileting and also affect their sleep, mood, and appetite;

> "the gastrointestinal symptoms impact quite literally on his independence and his quality of life. So, if they're bad, his quality of life is bad, he has less independence. If it's good, the independence comes back, the continence comes back, his quality of life comes back…….I mean, he can ride a bike, you know, he can't do that if he's incontinent and in nappies" [Interviewee 3]

Recurring vomiting after meals upset one child and they expressed this by scratching the parents during the vomiting episodes. This behaviour stopped completely when the vomiting was controlled for four months by medication.

Mental health effects

Parents reported various mental health effects precipitated by their child's persistent GI symptoms. These included fear and anxiety about passing stools, anxiety about participating in certain activities, anxiety about wetting the bed, an impact on self-esteem, and episodes of unexplained crying.   At times when the child's GI symptoms were under control, parents reported a better attention span, and their child being happier and calmer.   Some children were quite self-conscious about their GI symptoms and parents felt it made it more difficult for them socially;

> "I think that's been the thing that, you know, with all the things that come with being an autistic person in a neurotypical world, I think this one (gastrointestinal symptoms), from a kind of social point of view, makes it harder for him." [Interviewee 1]

Two children with complex needs communicated via their behaviour that they were unhappy about their incontinence and having to wear a nappy. This was expressed in by pulling the nappy off and scratching the parents when they are changing the child's nappy.

Not all parents were sure about whether their child's GI symptoms affected the child's self-esteem as some children did not seem openly upset about their GI symptoms. Parents were careful how they approached the subject with their child, choosing language that was non-shaming and trying to prevent making the child anxious about their symptoms by not drawing attention to it.   Other parents were clear that their child's self-esteem had been impacted;

"I think (self-esteem) is something that he struggles with to a point anyway, being different and kind of figuring out where he fits in the world. But it's, I think it was especially hard for him to see his little brother could go to the toilet quite fine and not have those issues with his tummy and things like that, but for him it was a big thing and it's quite an embarrassing thing." [Interviewee 10]

Physical health effects

Parents reported several physical health effects precipitated by their child's persistent GI symptoms including damage to teeth from recurring vomiting, difficulty maintaining an adequate body weight for their age and height, and hyperactivity.

"He had five teeth removed last year and his teeth turned black from the vomiting and his new teeth are just coming in and we really, really want to protect those (adult) teeth." [Interviewee 4]

Effects on schooling

In some cases, faecal incontinence had limited the choices for schooling and for one family it was a factor in choosing to home-school their child. Where the child attended a special needs school, parents reported more support for toileting issues and an acceptance of the occasional accident.

4.3.1.1.3 Impact on parents of managing their child's GI symptoms

Parents were using an array of lifestyle interventions and medications to manage their child's GI symptoms including;

- Giving a probiotic or fermented drink daily

- Organising regular exercise for the child

- Ensuring a good water intake during the day and not too much in the evening

- Daily administration of prescription medication

- Ensuring a diet with sufficient fibre, fruit and vegetables

- Establishing and maintaining a toileting routine

- Trialling exclusion diets (gluten, dairy or soya free)

- Managing the child's social routine to fit their bowel habits (e.g. taking them home after school to open their bowels while the parent is there to assist them, before taking them to a friend's house to play)

- Managing the child's anxiety (e.g. choosing to home-school)

Parents felt these measures helped with managing their child's GI symptoms and four parents described their child as no longer suffering GI symptoms.

> "He also has a really big meal of lentils every week, as well. It's kind of sweet potatoes, carrots and lentils, red lentils, and he has that every week, which really does support him. And we found we don't do that, he actually has to increase the medication." [Interviewee 3]

> "Before (they introduced lifestyle measures), it was either he was constipated or he was going because we were filling him in Movicol" [Interviewee 6]

Many parents reported benefits from incorporating specific 'toilet times' into the child's daily routine. These were times when the child was in a routine of sitting on the toilet with the opportunity to open their bowels. Most of the children had a regular time of day when they opened their bowels. Toilet times were easier for parents to implement with younger children who were at home for longer periods of time.

> "He can go an entire day without going to the loo, so for him I think it's had to become a bit more of a routine to ensure that he doesn't start getting backed up again and we end up in that situation again." [Interviewee 10]

For one child, a single enema enabled the child to establish a regular toileting routine after years of withholding;

> "since then (having the enema) he felt more able to start trying to go in the day and now he's got himself into a routine where every night before bed, he will go to the toilet." [Interviewee 10]

Toileting routines were not wholly without issues as some children spent long periods of time on the toilet, leading parents to build time-limits into the routine.

Ensuring a routine of regular exercise was reported as beneficial in helping to manage the child's GI symptoms. As well as the physical action of exercise on gut motility

[231], exercise is known to release endorphins and lift mood [232] so it is difficult to pinpoint the mechanism of the benefit from regular exercise.

Other therapies that parents reported as helpful in managing GI symptoms were intensive speech therapy to desensitise the child's mouth to food textures and oral motor therapy to improve biting and chewing of food. Parents reported that these therapies were instrumental in enabling children to expand their diet. Body awareness training where the parents prompted the child to recognise the physical signs of needing to go to the toilet, was also reported as helpful. A child with a co-occurring developmental co-ordination disorder, benefited from a frame around the toilet and a footrest for his feet, to enable him to feel stable and secure while sitting on the toilet. Although this worked well at home, it reinforced the child's reticence to use toilets outside of the home as they didn't have the frame around them.

For most of the children, it is their parents who are primarily managing the child's GI symptoms through observation and adjustment of lifestyle measures. However, this becomes more difficult as the child becomes older, becomes independent in their choices, and may choose not to comply with the advice given by their doctor or parent;

> "It's also she's got to an age where you've got to look at her compliance with what you want as well, because if, it does get difficult because she'll say well I'm really thirsty and I'll say but you're not supposed to drink after about 8 o'clock. She'll say 'but I'm so thirsty' and things like that and of course she can just go and get herself a drink without telling me, whereas when she was little she wouldn't have thought of doing that." [Interviewee 9]

Getting the child to engage with their treatment plan (where feasible) is always important but becomes central to success as they get older and more independent. At this stage, parents need their child to alert them to a change in their bowel habits so they can assist the child to adjust their lifestyle measures or medication dose;

> "What also has happened is, he's quicker at saying, I haven't been to the toilet for a few days, so we are quicker at noticing it so, getting his water intake up or saying right we need to have some more fruit and veg." [Interviewee 6]

4.3.1.1.4 Impact on the family

The children's GI symptoms had a significant impact on their family life. Any activities away from home (days out, going out for meals, holidays) were affected when the child cannot use a toilet outside the home or are in nappies at an older age and need changing while out. One child's recurring vomiting after meals had made it impossible to go away on holiday for several years or even to eat meals away from home. Additionally, strict diet requirements due to allergies, sensory issues or rigidity about food choices, also affect the family's ability to eat as a family ;

> "he is very particular so it's not just, pasta with cheese sauce, it has to be dried pasta as opposed to the fresh, it has to be white. It has to be the cheese sauce from Co-op, not from Asda and the pizza has to be the Marks and Spencer's Pizza, not the Asda or the Co-op, so it he's very, it's very limited. It's very difficult sometimes." [Interviewee 10]

These issues limit the things they can do as a family and leaves the parents trying to manage the often-conflicting wishes of siblings and the needs of their autistic child with GI symptoms;

> "If he's impacted bowel and suddenly has this awful explosion of diarrhoea, just randomly anywhere, well obviously we can't go out anywhere. So it's affected us socially that we just have been, literally housebound when it's really bad" [Interviewee 3]

> " he's on the toilet and there's just nothing you can do, if he needs to go. But we are much better at now, 10 minutes and then you have to get off. But if he's really struggling (with constipation) he'll do 10 minutes and get off but then 5-10 minutes, he'll be back on again, so it can be restrictive in the fact of going out, if he needs to go, we're sort of, we're just late and.." [Interviewee 4]

The autistic child with GI symptoms often needs more of the parent's time and attention for help with toileting and advocating for the right support for GI issues at school. Parents report having to do more laundry and the financial strain of having to buy night-time pull-ups and extra pyjamas, underwear, clothing and bed linen. Some parents reported that when GI symptoms affect the child's sleep, this can disturb the sleep of the rest of the family as well;

"Or, up all night, really loud because he is, when, at night, particularly actually, when there is discomfort it doesn't always present in a neurotypical way." [Interviewee 3]

4.3.1.2 Sub-theme – Managing GI symptoms is a source of stress for parents.

The children's GI symptoms and related pain were a source of stress for siblings and parents. Parents found it traumatic and very upsetting to see their child in pain due to GI symptoms;

> "when he needed to go he would cry and cry and cry and I'd have to hold on to him, and I'd rub his back and sometimes it would take hours. And then it would all come out at once. It would always be loads of it and it would be a whole horrible traumatic experience for him" [Interviewee 10]

> "it's just very, deeply distressing to all of us and his sisters, to see him in pain and crying, and like I said, when it's really bad biting on his finger, struggling in the toilet, you can hear him pushing." [Interviewee 3]

Parents found managing their child's GI symptoms stressful, particularly when away from home, but also at home when managing a child vomiting after every meal or regularly wetting the bed. Unreliable control of symptoms by medication was a source of stress for the parents and when the medication stopped working it seemed more stressful having had a break from the child's GI symptoms. Conversely, finding a reliable treatment that consistently controlled their child's GI symptoms reduced parent's stress. The long-term effects of the child's GI issues and their treatment, on the child's physical health was a source of stress.

> "he was vomiting for two hours after every meal , so six hours a day or vomiting, it was really, really horrendous. I got really, really bad. He was incredibly thin and he's like, it was really scary, wasn't it? Well he couldn't keep any food down so it was it was really bad." [Interviewee 4]

Parents were concerned about giving their child daily prescription medication for long periods of time, often with no understanding of how or why the medication was working or how long their child would need to be on this medication. Side effects of medication were a concern;

> "some of the tablets she's taken for her bladder have…one of them was terrible, it completely changed her character, she became very depressed and upset when normally she's quite a happy child." [Interviewee 9]

There was a financial impact in managing incontinence which could put pressure on family finances. Parents worried about the implications of unresolved GI symptoms becoming greater as their child progressed to secondary school and beyond and the child and their friends became more aware. They worried about the effect of symptoms such as flatulence and incontinence on their child's self-esteem, social acceptance and friendships. Specific events in the future (school journeys and sleepovers) where their child's GI symptoms might be difficult to manage were a concern;

> "When we're at home, if he gets some poo in his pants, he'll just say and we just deal with it. Now if he does that in front of his peers or he's smelly, or anything else like that, it needs to be dealt with sensitively." [Interviewee 1]

Parents worried about whether their child would need to take medication or live with a GI condition all their life. They wanted to know the future strategy for managing their child's symptoms in the longer term and had been unable to get this information from their healthcare providers.

Uncertainty about their child's symptoms was a source of stress for parents – not knowing why their child was crying and upset and being concerned that their child might be suffering GI symptoms without them being aware. Receiving a diagnosis of a GI condition did not always relieve stress, especially where there is no treatment for the condition.

Some parents found it isolating managing their child's GI symptoms on their own when there was nobody to talk to with the same experience. Parents also found it upsetting when pain-related behaviour was not recognised as such by staff at the child's school but was seen as bad behaviour.

In summary, persistent GI symptoms are impacting the child and family in a multitude of ways. GI symptoms are remaining unresolved for many years causing families to make difficult decisions (like home-schooling) and are a source of stress for parents.

4.3.1.3 THEME 2 – Understanding the nature and severity of gastrointestinal symptoms in autistic children is complex and multifactorial

4.3.1.3.1 Early onset of GI symptoms

The children's GI symptoms started when they were an infant or toddler. Early symptoms varied and included constipation with infrequent evacuation and hard stools that were difficult to pass, loose stools, odorous wind, vomiting, colic and silent reflux. Other early observations were that their child was a very restless baby who only seemed comfortable when being cuddled, frequently arched their back, would scream all day and night and was frequently ill.

> "he just was very, very, unwell and he had really explosive poos and diarrhoea and constipation and it would just go in horrible cycles like that from the moment he was born, really, and he would scream at night and hold his stomach and kind of bend over and just be really uncomfortable at night." [Interviewee 3]

> "From a baby she suffered really bad wind and colic and was quite disturbed by it for a long time." [Interviewee 7]

Most parents could not identify a precipitating factor for their child's GI symptoms starting. For those that could, the introduction of formula milk and solid food precipitated bloating in one child and a traumatic experience of dental treatment was a possible trigger for the start of constipation in another child.

For all parents, toilet training their autistic child was very difficult and took an extended time;

> "we take him to the toilet multiple times in the day, we sit him down. We've taken him out of nappies, we don't put him in nappies anymore. He hasn't been in nappies for a year. He is in underpants, which typically get soiled because he doesn't know, he can't, he's not toilet trained yet for number 2." [Interviewee 11]

This reflects research that indicates that around half of autistic children are not toilet trained by age 4 years [233]. Parents sometimes related this to constipation and the lack of sensations of needing the toilet;

"in terms of her potty training and her feeling of going to potty has also been better. We haven't had accidents for a long time now, and that's because she can actually feel her proper sensations yes, which we struggled with for a long time. So that is one of the problems when we had constipation and she went back in potty training because she actually couldn't feel the sensations and her tummy was kind of stuck there. [Interviewee 8]

There were added complications in unravelling the reasons for toileting resistance where the child was non-verbal. Parents commented that they were unsure whether their child understood what was expected of them during toilet training.

4.3.1.3.2 Unusual presentation of GI symptoms
Parents commented that their child's GI symptoms did not exist in isolation and there was a significant sensory aspect to them. The following behaviours were stated as indicators of GI distress;

- Jumping up and down when constipated and withholding a bowel movement

- Very upset and crying

- More distracted when GI symptoms are worse

- Self-injurious behaviour e.g. biting on their finger

- More meltdowns, being cross, frustrated, or aggressive

- Sleep disruption, restless and not sleeping

- Autism becomes worse when GI symptoms are worse

- Other conditions such as ADHD, Tourette's, learning disability become worse when GI symptoms are worse

- A strange smell to the child breath

Quotes regarding the presentation of GI symptoms;

"Yes, because you can see him when he struggle (to open his bowels), he will jump on the same space without movement. I mean he will stay still and at the same time jumping, kind of holding it, which mean probably he has some pain or he felt very uncomfortable." [Interviewee 12]

"So when he is experiencing intestinal pain, he will bite on his finger, he will jump up and down, he will get really upset, as well as being restless at night and not able to sleep, definitely. " [Interviewee 3]

"sometimes there's this misdiagnosis of GI symptoms, because I think the assumption is a neurotypical one, that the pain will always be expressed as crying and sometimes it is, but sometimes it **really** isn't, it's kind of more autistic behaviours; stimming, loudness, that sort of thing" [Interviewee 3]

" And, obviously having tummy aches is uncomfortable anyway and then because of his emotional regulation issues, any kind of discomfort seemed to be magnified emotionally so we'd see more meltdowns and more upsets" [Interviewee 10]

"When he wasn't vomiting there was no aggression at all." [Interviewee 4]

4.3.1.3.3 Complex inter-relationships with emotions, mental state and sensory issues

Most of the parents reported that their child did not use toilets outside of the home, especially for bowel movements. This was partially related to sensory issues and anxiety but was sometimes due to a previous traumatic experience. As a consequence, children were withholding at school and emptying their bowels immediately on getting home from school. If the child felt the need to open their bowels when they were away from home this often resulted in agitation and distress.

"I think, as much as it is the, kind of physical issue, it's very much a sensory, anxiety thing around toilets as well, which is difficult for him." [Interviewee 1]

"If he goes somewhere else, he won't use the toilet. So we've had an occasion, for example, when we were out, we were like a beauty local beauty spot and it was clear he was desperate for poo and he had poo in his pants and he would not sit on the toilet." [Interviewee 1]

Parents reported an inter-relationship between different GI symptoms and also between emotional state and GI symptoms in their children: They observed that

constipation worsened incontinence and reflux, and that anxiety worsened constipation; suffering GI symptoms led to aggressive behaviour or being upset; and being happy and sleeping well, helped with GI symptoms.

> "Because he was a lot calmer during (covid) lockdown, you know a lot of his anxieties went because he was in the house and managing it all a bit better. So, knowing he was going back to school, that, obviously he's a bit more constipated at the minute." [Interviewee 6]

> "..with my son, everything in a way stems back to anxiety. You know, and then the more we can kind of ease his anxiety, the easier everything else is, everything falls into place a bit easier." [Interviewee 10]

> "if he's constipated, he cries and he can get quite cross and upset, quite frustrated. Um, and then afterwards he might still feel a bit upset if he, if the bowel movement isn't satisfactory. So if he's constipated, it will be just quite an upsetting experience for him. If he's feeling OK, then it's all fine: He'll come, he'll sign toilet, he'll go and he'll be fine." [Interviewee 3]

Parents identified several triggers for the onset or worsening of GI symptoms;

- Anxiety was a trigger for the onset of diarrhoea and for the worsening of constipation

- A change in routine could exacerbate GI symptoms or cause the onset of new symptoms like reflux

- Certain foods like cow's milk, gluten, and dairy foods in general, were reported as triggers for constipation

- A lack of exercise exacerbated constipation

- Going to school increased the incidence of stomach ache

- Allergies were a trigger for nausea and stomach ache.

Parents also identified the well-recognised triggers for constipation of insufficient hydration, not enough vegetables and fruit in the diet, and missing a dose of laxative medication.

4.3.1.3.4 Parent uncertainty about the severity and nature of their child's GI symptoms

Most of the parents were unsure about the specific nature and severity of their child's GI symptoms. This led to the following issues;

- They are concerned that GI issues may make the child feel unwell and that the parent is not always aware

- Parents were sometimes reluctant to seek professional help for their child's GI symptoms as they couldn't confidently describe the symptoms or answer questions about the symptoms

- On the flip side, one parent reported that they were taking their child to the GP for reflux because they knew their child couldn't reliably report whether it was causing pain

- Difficulty distinguishing between ongoing GI discomfort and a new source of pain (e.g. toothache or earache). Resolving GI issues had helped one parent understand when their child is distressed and interpret the possible cause;

"so every time she cries it's a bit of a guessing game and sometimes it is really related to something but that's one thing that we can say that it's not tummy and that it's something else."[Interviewee 8]

Complicating factors

By a process of induction, we identified six factors which contribute to parents' difficulty in understanding their child's GI symptoms;

1. Anxiety

Anxiety about doctors may affect the child's communication about GI symptoms. Parent did not want to make child anxious by asking them questions about their possible symptoms;

"Or if I ask him if it hurts, he then looks a bit worried, like it should hurt, and then we get things like "My tummy's all over the place", it's this that and the other, and he's kind of a bit suggestible."[Interviewee 1]

2. Child's verbal ability

Limited language skills and understanding can limit the child's ability to alert parents to pain or to answer questions about GI symptoms;

" Yeah, if he suddenly starts crying, I don't know whether his head is hurting or whether his stomach is hurting. Unless there is a physical wound, there is no way of telling what's bothering him." [Interviewee 11]

3. Child's sensory issues

Sensory processing problems can make it hard for a child to identify pain or discomfort and to understand the signals for needing the toilet;

"I do sometimes feel like maybe he's still lacking the sensation because this was another thing we were concerned about is his sensory experience and whether he felt the need to go to the toilet and was deliberately withholding, or whether he just never felt, had the feeling and that's why he was withholding. And it was very hard for us to get a clear answer from him," [Interviewee 10]

"when you're out, (and) she wants to go to the toilet either for poo or wee, but then if you wait a little while, because you're nowhere near a toilet, then when you get near one she says "oh I don't want to go now"…… she's either not reading it correctly or her body's not giving the correct messages." [Interviewee 9]

4. Unusual presentation of pain or discomfort

Emotional dysregulation caused a magnification of the emotional expression of GI discomfort making it difficult to accurately assess the severity of symptoms;

"because of his emotional regulation issues, any kind of discomfort seemed to be magnified emotionally so we'd see more meltdowns and more upsets."[Interviewee 10]

5. Child's right and desire for privacy

Parents are less aware of, and less knowledgeable about their child's GI symptoms as their child gets older and toilets independently.

6. Autism inertia and difficulty with transitions

Some parents were unsure why their child was not going to the toilet and consequently soiling. A possible contributary factor mentioned by parents was that their child found transitions difficult and so could not break off from what they are doing to use the toilet.

4.3.1.3.5 Overall ranking of GI symptoms

Parents were asked to rank their child's GI symptoms in comparison with other day-to-day challenges their child faced. Not all parents felt able to do this, but of those who did, four rated it as either the top priority or the second highest priority. Two of these children were still suffering significant GI issues and had a co-occurring learning disability. One of these parents explained that although their child's GI symptoms ranked equally with the challenges of autism and learning disability, it was the GI symptoms that they felt they had been left to sort out on their own;

> "they are interwoven and inseparable really (autism, learning disability and gastrointestinal symptoms), and so the GI is just as important as all the others. It's the kind of, it's the one that's always forgotten and for me it's just so important. But it's the one that is the missing bit, I find, that you end up searching to sort out yourself, because if he's in pain and not feeling well, all the other, all the other diagnoses becomes so much more pronounced. [Interviewee 3]

Others ranked GI issues as middle-to-low with issues such as social communication, anxiety, demand avoidance and awareness of danger ranking as higher. One parent whose child's GI symptoms had resolved, commented that this enabled them to prioritise helping their child with other challenges such as speech and communication;

> "I feel like we have got the groundwork sorted and we have to actually work on the activities." [Interviewee 8]

4.3.1.4 THEME 3 – Access to healthcare services for autistic children with GI symptoms is variable and often limited, with diagnostic overshadowing

There was a lot of variation in the type and level of support and services experienced by the participants. For the majority of participants, the only health professional, if any, regularly involved in the management of their child's GI symptoms was their GP and this was often just for repeat prescriptions of medication.

4.3.1.4.1 Pathways experienced through NHS healthcare

The pathways through the NHS healthcare system varied significantly from one participant to another (see Figure 4-1). Two children were under the care of the Continence Team at the time of the interviews and their experience was very different to each other. One parent had regular 6-monthly phone calls with the Continence Nurse and found these very helpful in offering them reassurance about their approach and for offering ideas for different ways of doing things. The other parent was having monthly phone calls with the Continence Nurse who had been unable to meet them in person due to Covid-19 restrictions. This family were seeking help to toilet train their young child for bowel movements and felt they had gained little benefit from the phone calls. A third participant had support from the Continence Team in the past but was no longer under their care. Other participants were under the care of a urologist and a neurologist. Only four out of the twelve children had been seen by a paediatric gastroenterologist. This variety of pathways may reflect the complex nature of GI symptoms and their interplay with urinary issues, but parents found it frustrating and confusing.

Figure 4-1; Pathways through NHS healthcare

GP

First point of contact for GI symptoms or autism. Follow-up contact for repeat prescriptions or suspected infections

Paediatrician

Referred around age 3-4

Dietician
(3 participants)

If parent wishes to try a special diet

Paediatric Gastroenterologist*
(4 participants)

Neurologist
(epilepsy)
(1 participant)

Prescribed Omdansetron that reduced child's vomiting

Continence Team
(3 participants)

Service accessed around 7-8 years and out of nappies.

Occupational Therapist
(1 participant)

Referred by Continence Team as child was unstable on the toilet due to sensory perception issues

Urology at Children's hospital (1 participant)

Service accessed around 10 years old after several years with Continence Team not resolving symptoms.

* The age at which the child saw a paediatric gastroenterologist varied greatly with the oldest age being 9-10 years

4.3.1.4.2 Limitations of the professional approach

Several parents expressed a feeling that they had no choice but to manage their child's GI symptoms on their own.

> "We're pretty much on our own on this" [Interviewee 11]

> "I don't really believe anybody can help, so there's definitely that" [Interviewee 4]

> "a lot of the time she doesn't take the Desmomelt (medication) and I think why am I bothering really because it's not doing anything" [Interviewee 9]

By a process of induction, we found that the following things contributing to the parents' feeling of being left on their own to manage their child's GI symptoms;

- A health professional saying there was nothing else they could do for the child's constipation and faecal incontinence when the standard treatment with osmotic laxative didn't work

- Lack of active follow-up by the health professionals

- Long time-spells between appointments with health professionals and no way to discuss their child's symptoms when the symptoms changed between appointments

- Not being able to access services due to age restrictions

- Reasonable adjustments not being made which made it very difficult for the child to attend clinic consultations or get tests done

- Getting answers to questions through the NHS was cumbersome and time-consuming

- There seems to be a lack of a patient-centred multidisciplinary approach which leaves some symptoms unaddressed: For example, constipation

affects urine incontinence, but the children seem to either be under the care of only a urologist or only a gastroenterologist

- An apparent lack of expertise in the NHS to help their child

One family's story illustrates how parents feel left alone – their baby was being exclusively breastfeed and had not passed a stool for 10 days and then passed a very large silver-coloured stool (which the parent showed to the GP). Their GP was unconcerned and offered no practical advice and did not follow-up with the family to see if it had resolved. The child continued to struggle with painful constipation, but the parents tried to manage this at home and did not seek further help from the GP. Seven years later, the family had moved area, and the child had an introductory consultation with a paediatrician at the Autism Centre in the new local hospital. Again, the parents mentioned the child's severe constipation and faecal incontinence but were told that health services would not intervene in toileting until the child was at least 8-9 years old. The family has since chosen not to involve many professionals in the child's GI issues, but have resolved their child's constipation using diet changes, a probiotic and regular toileting routines.

4.3.1.4.3 Diagnostic overshadowing

Some parents felt that there was an unwillingness by health professionals to investigate, diagnose or treat GI symptoms in their autistic child, as it was regarded as part of autism.

> "we were just again fobbed off – this is autism, you know, he's going to have these issues, he's going to have these problems and not, 'How can we help? How can we make him feel comfortable? How can we actually give advice and try and treat it?'" [Interviewee 3]

An example of diagnostic overshadowing was one child with a history of severe silent reflux and vomiting from birth, with severe eczema following weaning. They were diagnosed autistic at age 2 years and were not allergy tested until age 6 years. Removing allergens from this child's diet resolved the eczema but they continued to suffer with painful bouts of diarrhoea and constipation and the child was eventually diagnosed with immature and impacted bowel at age 9 years.

145

"And I took him to one paediatrician who said that's just autism, autism goes together with gut issues, and I thought that's not a satisfactory answer. And then I took him to another paediatrician, whose daughter happened to be coeliac, and she listened to my, well, I explained that my son symptoms and he was tested, and he was diagnosed with coeliac. So it's lucky or just I was persistent that we got a second opinion really on that." [Interviewee 3]

It may be notable that only 4 of the 12 participants had received any GI investigative procedures; for two of these, this happened before their autism diagnosis, and the other two had complex co-occurring conditions as well as being autistic. One parent whose child's GI symptoms were investigated, said they were very clear with the paediatrician that they wanted any medical reason for their child's constipation investigated because they feared that it would just be put down to autism.

4.3.1.4.4 Parent confidence in managing their child's GI symptoms

Parents were asked whether they felt confident in managing the child's GI symptoms day-to-day and the differences between those answering yes and no were analysed.

Confident in managing their child's GI symptoms

These parents were finding the diet and lifestyle measures, medication and toileting routine manageable.

Things that helped to make the parent confident are;

- Regular 6-monthly phone calls with the Continence nurse

- The child's symptoms were mild, and the parent is clear that they would go back to the GP if symptoms got worse

- Situational effects e.g. most of one child's soiling happened in the evening when the child was at home, making it easier to manage

Despite feeling confident, one parent expressed a feeling of guilt at not having taken her child to the GP for their GI symptoms.

Not confident in managing their child's GI symptoms

For these parents, they had not found a way to consistently control their child's GI symptoms in a predictable way. The medication did not work consistently so the child's GI symptoms varied in nature and severity. In some cases, many treatments had been tried and the parent, and also sometimes the child, had lost confidence in ever finding a reliable solution. Parents wished they could do more for their child but didn't know how. These parents all expressed a need for more information about treatment options and what could help their child.

4.3.1.4.5 Unmet needs in addressing their child's GI symptoms
Parents were asked if there were things they needed help with, regarding their child's GI symptoms. The answers of parents fell into two categories; addressing current issues; and a quicker, more efficient way to get answers from the NHS to their questions about their child's GI condition or symptoms

Addressing current issues

These are the current issues parents wanted help with:

- Help from professionals to achieve a specific goal, for example toilet training their child who is minimally verbal; guidance on safely reducing their child's medication while maintaining their continence; help for their child to achieve settled bowel movements rather than swinging between constipation and diarrhoea; help to reduce their child's odorous flatulence.

- Assistance on dietary issues for example, personalised advice on ways to expand their child's limited diet taking into account their severe food neophobia or their child's limited language capacity

Quicker answers to questions

Parents found it complicated and time-consuming trying to get answers from the NHS about their child's GI condition or symptoms. This was particularly an issue when symptoms changed, and it was a long wait until their next appointment with a health professional. Parents sometimes filled this gap by seeking information and support

outside of the NHS system. They reported that Special Needs schools were helpful in signposting services like the Continence Service. Webinars or books authored by autistic adults were helpful in enabling parents to understand their child's autistic experience, e.g. sensory issues. Parents read articles online and in printed media on topics related to GI symptom management including early behavioural interventions, special diets, and GI issues in autistic children. Parent support groups specific to their child's GI condition (e.g. Rumination Syndrome) were also a source of information and support.

4.3.1.4.6 Parent's feelings about the healthcare received for their child's GI symptoms
Good experiences

Some parents reported an overall good experience: Things that they reported as good were;

- integrated services that worked together (GP, Continence service and Occupational Therapist)

- a thorough investigation of GI symptoms in their child even if a resolution was not found

- good adaptation by the health professional in their approach to communicating with their child

- a collaborative approach by health professionals with parents

There was one instance of effective co-operation between different services, when the Continence Nurse noticed that the child was unstable on the toilet and made a referral to Occupational Therapy. This led to a home assessment and the child being supplied with a frame to go around the toilet to help them feel stable.

One parent reported an effective adjustment to the communication needs of the child: The consultant paediatrician took time to explain the child's GI condition to them in a manner which caught the interest of their child; helped them understand the importance of following the doctor's recommendations; and motivated their child to

follow the treatment plan. The parent reported that this was a real turning point in the child taking ownership of managing their GI symptoms;

> "the consultant was really good at seeing how my son was in the room, when he was talking to me. So, things were happening, and he obviously took note of all of that, so when it came to giving the information, he then used some of the things that had happened beforehand, to get a hook in to talking with my son." [Interviewee 6]

> "It was really the way the consultant dealt with it, I think, was a turning point for my son, for both of us really, but particularly for my son, to understand what was going on and why it was happening and what he was doing to his body and how it would put itself right." [Interviewee 6]

Parents reported three instances of health professionals working collaboratively with them;

- GPs supporting parents to trial a special diet for relief of GI symptoms in their child by making a referral to a dietician

- A GP supporting parents trialling a special diet for relief of gastrointestinal symptoms in their child by doing a blood test to check for nutrient deficiencies

- A GP signposting parents to look for a clinical trial of a probiotic to help the child's treatment resistant constipation and soiling

Difficulties experienced

Other parents reported a less than good experience: Things that they reported as problematic for them were;

- Fragmented service

There appears to be a lack of continuity between different parts of the health service leading to patients being passed from one specialist to another, and the repetition of treatments that were not effective in the past.

- Long delays

The service was slow with long time spells between appointments, which was frustrating for patients, especially when the symptoms have persisted for years without resolution.

- Restricted access

There are restrictions on access to certain healthcare services, for example a minimum age for accessing the Continence Team.

- Diagnostic overshadowing

An autism diagnosis can be a barrier to a child being assessed for GI disorders; receiving a gastrointestinal-related diagnosis; or receiving treatment, when symptoms are assumed to be part of autism

- Reactive not pro-active

Parents report a lack of monitoring and pro-active follow-up by health professionals, which put the onus on the parents to manage the care plan for their child's GI symptoms.

- Not preventative

Generally, the service was poor at building a collaborative approach with parents for managing their child's GI symptoms; this missed an opportunity to act preventatively rather than being crisis-led.

- Lack of reasonable adjustments

Services were not accessible to this patient group in their standard form and reasonable adjustments were mostly lacking across all parts of the service, causing notable stress for parents and children.

Quote regarding Fragmented service:

"The Evelina obviously only give you a prescription for a small amount of medication, and then they tell you to go to your GP. So then you go to your GP and ask them if they can give you a prescription, they give you the same small amount, so you're always getting small amounts of medication and having to ask for renewals of the prescription"
[Interviewee 9]

150

Quotes regarding Long delays:

> "The lead time to get access to these services is quite long. I mean OT we've needed, we've tried to get OT for the past 2 ½ years." [Interviewee 11]

> "so I had to stop that (medication) after 2 weeks but you still don't get an appointment for 6 months. And one time I thought I hadn't heard from them for a long time and it turned out it was a year since she'd had an appointment with them" [Interviewee 9]

Quote regarding Restricted access:

> "The paediatrician said the tummy aches were due to infrequent opening of the bowels but didn't offer any help or support; they said they wouldn't intervene with toileting until he was at least 8 or 9 years old, so we weren't really offered any help or support on that." [Interviewee 10]

Parents were concerned about the long-term use of osmotic laxatives with their child without any regular review by health professionals. They wanted to know what the long-term plan was and whether their child would need this medication for the rest of their life. Where parents had found a dose of osmotic laxative that worked well for their child, they generally seemed less concerned about giving the medication long term. Other parents were unable to find a dose that consistently cleared their child's bowel without causing loose bowel movements and soiling, meaning that their child was yoyoing between constipation with a loaded bowel, and diarrhoea with soiling.

4.3.1.4.7 Reasons for not seeking NHS healthcare

Three parents had not sought help from the NHS service for their child's GI symptoms. The reasons for this included;

- The child's symptoms were not severe enough and did not seem to cause the child distress or discomfort

- The child's GI symptom was present in the parent so was regarded as normal

- Parental time constraints

- Feeling unable to adequately describe the child's symptoms to the doctor as the child was minimally verbal and couldn't answer questions about the site, nature, and degree of pain

- A previous traumatic experience at a healthcare appointment led to a degree of reluctance to seek medical help in some parents, and anxiety in the children about any medical appointment

- Constipation was not considered by some to be a medical condition but rather something to be managed at home

Three parents had consulted a private health practitioner about their child's GI symptoms, in conjunction with using NHS services. The reasons for using private services were;

- To try and understand their child's GI symptoms better

- To see a health professional who had more time to treat their child and take a really full case history

- To engage a health professional who was more readily contactable when the child's symptoms changed

Parents were generally nervous about using private medical services for their child's GI symptoms;

> "I can see that there would be links between what's going on in your gut from everything I was reading and what's going on in your brain. But the fact that nobody is actually, it feels a bit shotgun, and you don't know who you're going to, what you're doing and what you're messing with. So I backed off at that stage." [Interviewee 2]

> "No (did not sought help for gastrointestinal symptoms privately). Because we didn't quite know what we were looking for. Is it constipation? I mean see, umm, I don't see that as a, okay we don't see that as an identified illness." [Interviewee 11]

4.3.1.5 THEME 4 – Reasonable adjustments to the current NHS service are needed
to reduce child and parent stress

As mentioned in Theme 3, NHS healthcare services are not easily accessible to this patient group in their standard form and reasonable adjustments are mostly lacking across all parts of the service causing notable stress for parents and children:

"It was so difficult, they'd changed the ways of getting blood tests and schools were being, you know, because he'd had time off school due to autism, schools were sending like welfare, educational welfare to me, so having one day off and going to get a blood test involved just turning up at the hospital and you never knew whether they were going to accept you to do it" [Interviewee 5]

"I remember one appointment, he was so scared that he just screamed the entire time. He took off all his clothes and then he bit me on the neck and between me and the doctor we could not get his teeth off me. And in the end, I said to her, could you just help me get his pants and trousers on, because then I can take him outside and I think he'll calm down. So, with his teeth still in my neck, we both had to struggle to get his clothes on, and I think actually, if that had been during the pandemic and we had to do just a phone call or video call, we would have avoided that whole distressing situation for him, because, really he didn't even need to be there." [Interviewee 10]

"Briefly, this is what we have to do when we go to the doctors. We have to go, and I have to go into reception, I have to leave him in the car because he absolutely hates going in the doctors because he knows the doctors is, there's nothing motivating, reinforcing or nice about it. And yeah, and then I have to go and tell them that I'm there, then I have to go and sit in the car and wait with him, and then they call him in, and then usually they say they're ready to see him and yet they make us wait even more, in the waiting room. And then he bounces about the room like a pinball while they try and examine him. It's just so horrendous, and he just signs "finished" the whole time because he just doesn't want to be there. It's just horrendous. It's horrendous." [Interviewee 3]

A process of induction identified three categories for the reasonable adjustments requested by parents; i) communication, ii) consultations and iii) resources.

4.3.1.5.1 Communication

All communication with the child whether verbal or otherwise, needs to be tailored to their abilities and learning style (e.g. visual, auditory or written) and it may need to take account of their interests to achieve their engagement. Parents also reported that instructions for the child's involvement in treatment can be easier for the child to understand and comply with if it's broken down, for example replacing guidance to eat five portions of fruit and vegetables every day with eat 2 portions of fruit and vegetables with each meal.

The families need accessible information before a clinic visit to prepare their child and reduce anxiety. This may need to include pictures of the building, room, and staff, as well as details about the consultation itself e.g. whether the child will need to remove any clothing, lie on a bed and whether the doctor will physically examine them. A productive doctor-patient relationship should be built on trust and to promote this, parents wanted clinicians to adjust their communication style to the child's ability and interests. Some parents reported that this had improved their child's engagement with the treatment plan, and that having an understanding their GI condition and treatment plan, improved their child's self-esteem. Other parents reported that their child had lost confidence in the doctors ever being able to resolve their GI symptoms and consequently their treatment compliance was poor. Maintaining the child's engagement and confidence in the treatment became increasing important as the child became more independent in self-care. If part of the treatment plan requires a child to change their habits (e.g. drinking 8 glasses of water daily), parents felt that the health professional should explain to the child how it will benefit them, to aid motivation and compliance. Associated with this, health professionals should keep in mind that autistic children may not be aware of or motivated by social norms (like not passing wind in the classroom).

 The majority of parents wanted more information about their child's GI condition to enable them to be pro-active and prevent symptoms where possible. This included understanding the causes and triggers of their child's symptoms and the long-term plan for treatment.

4.3.1.5.2 Consultations

Parents expressed a need for a more wrap-around service for the family that would help with all aspects of managing their child's GI symptoms including gaining treatment compliance with older children. They wanted more frequent appointments with healthcare professionals when trying to resolve a chronic GI condition, but not necessarily with the consultant: One parent reported having regular appointments with a specialist continence nurse which gave them confidence in managing their child's GI condition and reduced stress. Parent's wanted clinicians to listen more to patients and their parents and wanted to work collaboratively with health care professionals to manage or resolve their child's GI symptoms. One aspect of this was the need for flexible treatment approaches that considered autistic children's sensory hypersensitivity to taste and texture. For clinic appointments, a peaceful waiting area with reliable Wi-Fi was important to families to reduce child and parent stress. Some parents found telephone or video consultations less stressful for themselves and their child than attending in-person and would prefer this option going forward. Other parents felt that a physical examination of their child was essential as the child could not reliably communicate pain.

4.3.1.5.3 Resources

Parents reported a need for specific resources for autistic children who may be non-verbal and visual learners; may not be attuned to what is socially acceptable; and may not be motivated by the same things as their peers. Parent suggestions included videos about the process of going to the toilet using animation or cartoon characters, and social stories about societal expectation regarding GI issues. Parents wanted education resources or therapy to help their child understand the sensory messages from their bowels and bladder, which they felt were key to improving the child's continence and independence.

4.3.1.5.4 Opinions about video consultation for medical appointments

As video and phone consultations have become part of standard medical care since the Covid-19 pandemic, we asked parents how they felt about these for their autistic

child. The group of interviewees were split down the middle on whether this was helpful for their child or not, and through analysis we could not determine a characteristic driving this preference. There were three non-verbal children in the group and the parents of two of these preferred video or phone consultations and one did not. There were two autistic children with complex co-occurring conditions in the group and both families preferred video or phone appointments. There were four parents who reported that their child had a very stressful experience at a previous medical appointment and three of these preferred video or phone appointments and one did not.

Disadvantages of video or phone consultations for autistic children

Half of the parents were not comfortable with video consultations for medical appointments for the following reasons;

- it was too easy for things to be missed

- parents wanted the reassurance of a doctor seeing their child in person

- it would be difficult or impossible for their child to participate in a video or phone call

- the child would not get as much out of a videocall as an in-person

- the recommendations of the doctor would not be taken as seriously by their child;

  "(my son) sitting with that consultant is part of what changed his experience. I think just hearing it from me or hearing it on Zoom wouldn't have the same gravitas as it did" [Interviewee 6]

Advantages of video or phone consultations for autistic children

Three parents were delighted with the advent of phone and video consultations as taking their child to the doctor's surgery or hospital was fraught with difficulty and very stressful for the child and parent;

"..so now the system is you take a photo, you send it into the doctor, and you discuss it over the phone. Well, that's brilliant because it does, it means that he doesn't have the trauma of waiting and sitting in a doctor's room, and I'm able to take lots of angles of it on my camera and send it through. So I don't, I think in a way, this system is better, unless he needs a physical examination or unless they are specifically looking at his autism, for example, in a paediatric appointment. I don't see the value of it at all, it's just very traumatic. " [Interviewee 3]

Stated advantages or video or phone consultations were;

- a videocall would be less intimidating for the child

- it would be less stressful for the child to be in their safe space at home

- not having to travel to the hospital or clinic with their child reduces stress for the child and parent

- Avoids the stress of strange places and sensory overload for the child

- Video or phone consultations are easier to fit in the day as they take less time (no travel time) and its easier managing childcare for siblings

The remaining three parents did not have a single preference but felt that some medical issues required in-person appointments and others were suitable for a video or phone consultation.

Given the wide heterogeneity of children with an autism diagnosis, it is not surprising that a one-size-fits-all medical consultation does not suit all families. Overall parents felt there was merit in telemedicine and in-person consultations and preferred to have access to the different options so families could choose the type of consultation they needed. The nature of medicine dictates that sometimes a physical examination will be needed. Medical professionals should be aware of the stress in-person consultations may cause for the child and parent and enquire in advance about reasonable adjustments that may facilitate successful attendance.

4.3.2 The effect of home-confinement on autistic children's GI symptoms and the family experience of managing these

In answer to the question "How has Covid-19 lockdown and Covid-safe measures in schools affected the child's GI symptoms and the family experience of managing these symptoms?" our analysis revealed one major theme and one sub-theme;

- Covid-19 lockdown and Covid-safe measures at schools affected GI symptoms in autistic children but not in a uniform fashion.

  Sub-theme - Extra family time allowed a focus on the child's self-care and life skills

4.3.2.1 Covid-19 lockdown and Covid-safe measures in schools affected GI symptoms in autistic children but not in a uniform manner

There seems to be several interacting factors contributing to a change in GI symptoms in some of the children during Covid-19 lockdown. Home is a 'comfort zone' for most children and from the interviews, this also applied to the autistic children in this study. All the children were not able use a toilet outside of the home for bowel movements and many would withhold bowel movements while at school with subsequent discomfort. In contrast, during Covid-19 lockdown they were able to use the toilet whenever they needed. Consequently, some parents reported that their child's constipation improved during lockdown. For the children that were anxious in social situations, parents reported that their child was calmer, less anxious, and happier during lockdown as there was no socialising.

> "He's really happy at the moment because he hasn't been to school for six months. So he has been very different" [Interviewee 5]

> "Because he was a lot calmer during (covid) lockdown, you know a lot of his anxieties went because he was in the house and managing it all (constipation) a bit better. So, knowing he was going back to school, that, obviously he's a bit more constipated at the minute."

All the parents reported that managing their child's GI symptoms was easier at home, and some parents reported being more relaxed about their child's GI symptoms during lockdown.

(after lockdown) "his friends were coming round to see him so, you know, he suddenly felt that pressure again. I'd forgotten that we haven't had that pressure for a while" [Interviewee 6]

Covid-19 lockdown was a change in the children's' usual routines and there were restrictions on time spent outdoors and where you could go to exercise. Some parents reported that this had a detrimental effect on their child's GI symptoms and mood;

"when things change, or routine's different, then we get a lot more of that (faecal incontinence). And at the moment, because things, we've had so much changes (Covid restrictions and lockdown).… After the last couple of weeks he's got the most alarming reflux." [Interviewee 1]

Prior to schools shutting for lockdown and when they re-opened after lockdown, the children had to get used to new Covid-safe procedures. Not only was this a change to the usual school routine, but the new procedures also introduced extra challenges in the management of autistic children's GI symptoms. These measures had a uniformly negative effect on children's GI symptoms. School staff could not risk cleaning children up after vomiting or soiling and consequently children were sent home when this happened, causing some to miss a lot of school which impacted their learning. Water fountains were switched off in schools so children couldn't refill their water bottles and staying hydrated was difficult. It was more difficult for their child to access the toilet, as children were allocated to a specific toilet that could be a long way from their classroom. Additionally, children were only allowed to use the toilet at a set time during the day and this didn't necessarily fit with the child's needs.

"with the Covid thing she's got to go miles to HER toilet (at school) because they're not allowed to use each toilet – they have their own toilet now. So at the moment it's even worse really" [Interviewee 9]

For two children, the lockdown and subsequent return to school routine had affected their regular bowel habits and they were finding it difficult to establish a regular routine after lockdown finished.

"it's not settled again, when he goes to the toilet. We had to get him going in the morning (because of going back to school). It did change to the afternoon, it seems to have gone back to the morning again now. I think he likes to go before he goes to school, and if he doesn't then he's holding it until he gets home. And during lockdown he could go whenever you wanted so I think it got later in the day." [Interviewee 6]

Two parents felt the Covid-19 lockdown had a negative effect on access to healthcare for their child, including finding it difficult to see their GP, and appointments with the Continence Team being delayed by several months and being a telephone consultation rather than in-person;

"I think it would have been a lot more effective if somebody could come see him, or we could go see somebody and, sort of, this phone consultation is very, it's not effective" [Interviewee 11]

4.3.2.2 Sub-theme: Extra family time allowed a focus on self-care and life skills

With the whole family being at home during Covid-19 lockdown and regular clubs and activities being closed, this allowed some parents the time to encourage lifestyle changes that could help manage their child's GI symptoms including; toilet training; expanding the range of foods their child would eat; or establishing a regular routine of exercise. One parent got their child involved in cooking the family dinner with great success;

"So one of the things that we did through lockdown was that he had to cook a meal and cook a sweet treat once a week, so that he would have different meals that we knew he could cook, and trying to get him more interested in food, I think that definitely helped (talking about expanding the diet)." [Interviewee 6]

4.3.3 Lessons from the VIVO-ASD study and barriers to participation in clinical trials

In answer to the question "What can we learn from the experience of participants in the VIVO-ASD clinical trial that could inform the design of future clinical trials to make them more accessible and acceptable to families with an autistic child with GI symptoms?" our analysis revealed 2 major themes;

- There are barriers to involvement in a clinical trial for autistic children and their parents.

- Parent's experience of participating in the VIVO-ASD clinical trial with their autistic child could help inform future autism gastrointestinal research study design.

4.3.3.1 THEME 1 – There are barriers to involvement in clinical trials for autistic children and their parents

We explored how participation in clinical trials could be made easier for families with an autistic child. We asked parents to give their opinion on various aspects of a theoretical research study to understand potential obstacles to participation in autism intervention research for GI symptoms.

4.3.3.1.1 Suitability of outcome measures

Biological tests were challenging in general, and most parents would not consider a clinical trial that involved blood tests. Those that would consider it, felt that they would only be comfortable with a maximum of three blood tests over the course of a study and even then, it would be a struggle for their child. Collecting urine or stool samples from children would not be a barrier if this could be done at home and is either infrequent (e.g., monthly) or for a short period of time (e.g. a week). All parents felt collecting urine or stool samples from their child in a clinic situation would not be feasible. Diaries of symptoms (e.g. stool diary) can be problematic when the child is unable to report what happened at school or where several carers look after the child over the course of a week. Parents felt they would need a reminder on their phone to complete daily reporting of any kind and felt that the process of reporting should be simple and quick, with an 'other' free text box as things are rarely simple with an autistic child. There was a preference for a phone app or being sent a weblink by text to enable daily reporting. The advantage of a web application is that multiple carers can report for the same child.

4.3.3.1.2 Other aspects of study design

A high number of visits to the research centre that caused their child to miss school, was identified by parents as a barrier to participation, but if appointments could be scheduled in school holidays, at weekends or after school this was not such an issue.

The issues regarding clinic visits for medical appointments identified in Theme 4, also apply to research study visits: new places and people are often stressful for the children and efforts should be made to reduce this by making reasonable adjustments. Parents were generally less keen on participating in a research study for a drug rather than a food supplement. The main concern with this was the possible side effects. It was also mentioned that any product to be taken as a liquid, powder or chewable should be bland tasting due to hypersensitivity to taste. Simplicity and clarity of their involvement was important to parents, and they wanted studies to be easy for them to follow and not take too much time of the their time.

4.3.3.1.3 Travel to the research centre

Travel to a research centre or hospital was not generally considered an insurmountable barrier. Driving was generally preferred to taking public transport, so a study centre with parking would suit most families. However, driving to a central London location was not practical due to traffic and congestion charges. All parents preferred a video consultation to complete assessment questionnaires but felt it was important to visit the research centre with their child at the start of the study to reassure themselves of the legitimacy of the study and for the child to understand the importance of their involvement.

4.3.3.2 THEME 2 – Parent's experience of participating in the VIVO-ASD clinical trial with their autistic child could help inform future autism GI research study design

The parents' experience of participating in the VIVO-ASD study can offer guidance for future research study design for treatment interventions for co-occurring conditions.

4.3.3.2.1 Main motivation for participation

The most common primary motivation for taking part in the VIVO-ASD study was to help their child with the discomfort or pain they suffered as a result of their GI symptoms. Consequently, the fact that their child was guaranteed to receive the real treatment product at some stage was an important motivator for participation. In parallel with this, several parents said that they were motivated by the chance for their child to try a probiotic as part of a hospital-based trial so they could see if it helped

their symptoms. Another motivation was being involved in good-quality research in the field of GI disorders and autism which they felt was an important and under-researched area. Parents hoped that other families would benefit from the results of the research.

4.3.3.2.2 Secondary gain from participation

Improving communication about GI symptoms

One of the unexpected gains from involvement in the study was the use of the Bristol Stool Scale and how that aided parent and child communication about bowel movements. Some families continued to use this after the study finished and they felt it would be useful in the future when talking to medical professionals about their child's GI symptoms.

Child's confidence and self-esteem

Parents took the opportunity of going to the research appointments at the hospital to have a day out in London or to meet family or friends in London. It gave parents an opportunity to have some parent-child time with just their autistic child and for their child to feel they were doing something different and special. Going to the hospital and meeting the researcher helped their child feel they were part of something important. The research visits were a nice reason to use public transport, and this gave their child the confidence of knowing they could manage this.

Family learning

Families learnt different things from their experience in the study including how much probiotic their child could tolerate and how far their child had come in overcoming their challenges.

4.3.3.2.3 Things that aided participation or staying in the study

Taste Test

Most parents reported that the Taste Test week at the beginning of the study aided their participation as it alleviated worries about whether their child would take the product. A few parents also mentioned that the taste test gave the child the choice to participate and gave them more agency in the decision.

Features of the treatment product

For most families, the powder format and mild taste of the product helped participation as they could mix it into a food or drink that their child liked and was familiar with. Some children were already familiar with powder medication because they were used to taking Movicol or Laxido. The majority of parents felt that if the treatment had been tablets or capsules, their child could not have taken part. Flexibility about when the product could be given (any time of day and with or without food), also helped participation as it could be fitted in with the child's existing routine.

Study design

The fact that their child would get the real product at some time, helped families continue in the study. Regular support calls every four weeks with the researcher helped the parents stay on track and feel confident to continue.   The simplicity and clarity of the study helped parents participate and the fact that involvement was not overly demanding or stressful helped them continue participation. Not having to wait long in the waiting area helped families stay in the study as this was very challenging for their child. It was mentioned that visiting the hospital at the start of the study aided the child's continued participation as they understood they were part of something important.

4.3.3.2.4 Suitability of outcome measures

The questionnaires used in the study (ATEC, Gastrointestinal History, ABC, Autism Parenting Stress Index) were considered by the parents to be relevant and not too onerous to complete.

4.3.3.2.5 Things that could be improved about the VIVO-ASD study

Supplying pictures of the hospital, the reception area, the clinic room, and the researcher, would have helped some parents to prepare their child for what was going to happen and reduce any anxiety. It was mentioned that the WIFI in the waiting area and clinic room was poor, and this caused some children some distress so better WIFI would reduce child and parent stress and make clinic visits easier.

4.3.3.2.6 Challenges encountered while participating

Research study visits

The study involved visits to University Hospital London which is in central London and many participants travelled there by train. Some children found travelling on public transport challenging and it was difficult for the family to manage. In contrast, other children enjoyed going on the train. The clinic room was a bit distracting for some children, particularly the presence of a sink. Attending the hospital had its difficulties for the children as it was a new place. One parent found it difficult to find the clinic room once at the hospital so meeting the participants at the entrance would have been better. Waiting to see the researcher was difficult for most of the children but this was a difficulty with unusual places and waiting rather than a particular aspect of the waiting area.

Issues with the product

The powder format was difficult for some children at first but then they got used to it. Despite the fairly bland taste of the product, some parents found it difficult to find a drink or food to hide the taste of the product and make it acceptable to their child. Parents have busy lives and remembering to give the product daily was mentioned as a challenge. Vivomixx has to be kept refrigerated and this was difficult for some parents as the boxes of product took up a lot of room in the refrigerator. The need for refrigeration also caused some difficulty when going away on holiday during the study. Side effects of the product were rare, and none were serious but they caused some difficulty when they happened. It was important for parents that they could contact the research team at any time when possible side effects occurred and get a prompt response.

4.3.3.2.7 Regular information channels

Recruitment to the VIVO-ASD study was more difficult and took much longer than expected, partly due to the poor response rate from our initial mailout to families supported by Caudwell Children. Consequently, it was necessary to find effective ways to publicise the study. We asked parents about where they regularly looked for information about autism and things relating to their autistic child to get an insight into

where to publicise a research study. Only two parents were registered with an autism research database, and I did not find this a good source for participants. Parents told us that they looked regularly at parent support groups on Facebook and received information regularly from local National Autistic Society groups and local Mencap groups. My experience in the VIVO-ASD study was that contacting these groups was a productive way to find potential participants. Other sources of information were the child's school, Autistica website and the Thomas Centre.

Based on this, productive places to publicise research studies for autistic children are via local Mencap and National Autistic Society groups and via autism-focused parent support groups on Facebook.

4.4   Limitations and strengths

The limitations of our study are that all participants are a subset of those who participated in the VIVO-ASD probiotic clinical trial which may introduce some selection bias. All participants lived in England and accessed healthcare via the English NHS. The interview questions were drawn from our experience with families during the VIVO-ASD study taking a pragmatic, real-life approach. As with other studies done during Covid-19 lockdown, parents' thoughts and feelings may have been affected by stressful events at that time. Although the researchers analysing the interview data endeavoured to be aware of their bias and to avoid that influencing interpretation, this possibility always remains in this type of research.

There are several strengths of this qualitative research. Firstly, the twelve interviews achieved data saturation as new topics were no longer evolving. Parents and their autistic children were consulted on the questions being asked which should ensure their relevance to families. The interviews were transcribed by one of the researchers and two researchers independently coded the transcripts and discussed and finalised them together. To get different perspectives, one researcher had a background in gastrointestinal physiology healthcare and the other has lived experience of autism and GI issues and clinical experience in nutrition. The Framework technique was used to analyse the interviews and a strength of this approach is that it develops an audit trail of the analysis. The participants included a mix of girls and boys and a range of ages, with a geographical spread across England, and a variety of GI symptoms. They

also included children with varying impact from their autism and participants with co-occurring conditions and minimal language. The results of the analysis were sense-checked by sharing the results with a mixed group of GI physiology colleagues.

4.5 Discussion

In our qualitative study we found that the children's GI symptoms started at an early age and were persistent for a number of years despite treatment. Research suggests that GI symptoms in autistic children are likely to continue into adulthood: gastroesophageal reflux disease (GERD) symptoms are common in autistic children [233] and there is also a notably increased prevalence of GERD symptoms and complications in autistic adults compared to non-autistic adults [234]. Likewise, the prevalence of GI disorders in middle-aged and older autistic adults was found to be 49.7% [235] which is slightly higher than median prevalence of 46.8% for any GI symptoms in autistic children [236]. In this study, parents reported that toilet training their autistic child was difficult and protracted. This reflects research that 57.2% of autistic children are not toilet trained by age 4 years [233], and toileting resistance is more common in pre-school autistic children (49.1%) than in similar aged children with developmental delay (23.6%) or the general preschool population (8%) [91]. That study identified the following factors as associated with toileting resistance in pre-school autistic children exclusively; lack of social motivation, constipation, delays in expressive language. Diarrhoea and lack of social awareness was associated with toileting resistance in both autistic and developmental delayed preschool children [91].

We found that managing their child's GI symptoms was a source of stress for parents. This agrees with the findings of Mannion and Leader 2023 [237] who found a relationship between parental stress and child GI symptoms. They also found that compared to parents of autistic children without GI symptoms, parents of autistic children with GI symptoms reported lower levels of quality of life; were less satisfied with their personal and social relationships; and reported lower levels of social support [237]. From our study we know that resolving the child's GI symptoms and/or a thorough investigation of GI symptoms reduces parents' stress. It may be that diagnostic overshadowing is affecting GP referral to GI specialists for autistic children

as only four of the twelve children in this study had seen a Paediatric gastroenterologist. Parents were happier with the service when different parts of the healthcare system collaborated to resolve issues affecting the management of GI symptoms in their child. They were also happier when the health professional took a flexible, open, and collaborative approach with the parent and child to resolving or managing GI symptoms. Finally, regular contact with a GI health professional eased parent stress.

Parents in our study reported a number of factors that they felt were interconnected with their child's GI symptoms. Analysis of the interviews and relevant research literature, suggests a complex, interwoven picture which appears to be different for different age groups [238]. Recent research indicates a bi-directional relationship between GI symptoms and internalising behaviour such that they simultaneously affect one another [97]. Therefore, it may be necessary for clinicians to treat both in order to resolve either GI problems or internalising behaviour such as anxiety. In our research, several parents reported a close connection between their child's state of anxiety and a worsening of GI symptoms and vice versa. Clinicians would be well advised to consider this potential relationship during assessment and treatment of GI symptoms in this patient group and also to inform parents of this potential relationship to aid management of their child's GI symptoms.

As discussed in section 4.1 chronic constipation can drive behaviour and vice versa [64]. The information given by the parents in this study suggests that the same sequence of events appears in autistic children but with different behavioural expressions. The parents in our study reported a number of behaviours that they recognised as indicators of GI distress in their child. This reflects findings in qualitative research from the USA that found parents relied on behaviour and bodily signs as indicators of GI distress in their autistic child even if they were verbal [53]. However, this can still leave parents confused about the specific nature and severity of their child's GI symptoms due to the sensory, perceptual and language challenges their child faces. It is unclear from our study whether this led to delays in seeking medical advice for their child's GI symptoms, although some parents did express a reluctance due to previous stressful experiences at medical appointments with their child or a lack of confidence that the doctor could help. A debate is perhaps needed in the

paediatric gastroenterology community about the merits of invasive investigations in some autistic children, particularly those who have extra challenges recognising and reporting pain. Currently there is a hesitancy about invasive gastrointestinal investigations in children, and it seems there may be an even greater hesitancy for those with neurodevelopmental conditions like autism. Considering how parents in this study have reported difficulty understanding how GI symptoms are affecting their child; not being sure whether their child is in pain or not; and not knowing what is behaviour unrelated to pain, it raises the question of whether the profession should consider invasive GI investigation in some cases. Certainly, there is a pressing need for a validated autism-specific bowel symptoms assessment tool, and this should include accessible resources to help the children themselves contribute to the assessment, even when they are minimally verbal.

The importance of reasonable adjustments to facilitate healthcare access for autistic children is underlined by the reporting of poor healthcare outcomes in autistic adults [44]. The message from parents in this study was that the standard medical practices failed to consider the sensory hypersensitivity of autistic children to taste, smell and texture of medication, and to take account of their child's anxiety about strange environments and physical touch or the possibility of sensory overload in a busy clinic environment. This reflects research looking at barriers to healthcare for autistic adults compared to non-autistic adults which found significantly greater difficulties for autistic patients in a number of areas including; difficulty summarising symptoms for the doctor; sensory problems with waiting rooms; anxiety affecting their communication skills; and difficulty with uncertainty when details about the appointment were not specified in advance [239]. Despite this, parents wanted to understand their child's GI condition and the long-term plan for treatment, and to work collaboratively with health professionals to resolve or effectively manage it. The need for adapted information before a clinic visit has previously been reported in the context of autistic adult patients attending their GP [36] and this study confirms this need in autistic children and adds specific details pertaining to children's information and communication needs. There has been some research which could inform reasonable adjustments for autistic children with GI symptoms: A study of improvement strategies for autistic adolescents undergoing endoscopy [240] reported some similar results to this study including requests for autism specific information to help prepare the young person prepare for

the procedure, and similar issues with noisy and busy waiting rooms. The same study presented recommendations from parents to improve the experience of endoscopy service including; training for care staff about the communications and sensory needs of autistic individuals; letting the patient and family wait in a separate quiet waiting room or a preferred space like in the family car in the hospital car park; and sending paperwork to the family for completion prior to their visit to reduce waiting time at the hospital [240]. Boston Medical Centre has a Autism Friendly Initiative [241] and uses an Autism Support Checklist which is completed in advance of a hospital visit and used to inform all staff of the specific needs and communication preferences of the autistic patient. The National Autistic Society (NAS) in the UK has developed a health passport for autistic people which can be completed online or downloaded from their website [242] This includes sections for communication preferences; how the person communicates pain; things that cause the person distress and things that make them happy. The experiences of the families in this study suggests that more use of the NAS health passport by families and clinicians is warranted.

Our findings regarding the appropriateness of telehealth for families of autistic children generally reflect the findings of Franz & Kelly [243]. The parents in our study were happier to take part in a telemedicine consultation when they felt there was not a need for a physical examination. Franz & Kelly (2021) asked parents about their willingness to participate in behavioural telehealth appointments for their child and the mean willingness score was 6.89 on a scale of 0-10 (higher score representing more willingness to participate). In our study, parents expressed advantages and disadvantages to both telemedicine consultations and in-person consultations for their child. Telemedicine obviously removes the difficulties of travelling with their child and some parents reported that their child found public transport or long car journeys challenging. However, where the child can communicate with the doctor independently, parents felt that attending an in-person consultation could aid the child's understanding and commitment to the treatment. Parents also expressed concerns about telemedicine consultations where their child was non-verbal or unable to accurately report pain: They felt that a physical examination was essential in some instances and given the communication difficulties and sensory processing issues commonly found in this patient group, there may be a lower threshold for this need.

In summary, GI symptoms have a significant impact on autistic children and their families, and this tends to increase at the child gets older. Despite this, there appears to be no consistency in GI services or the path to these services and autistic children are suffering with GI symptoms for protracted lengths of time. Consistent findings are that diagnostic overshadowing affects the GI health services offered to autistic children; there is seldom adjustment of services to enable accessibility for this patient group; parents feel left on their own to manage their autistic child's GI problems and this is a source of stress for parents.

We found that persistent GI symptoms in autistic children changed during the home confinement of Covid-19 lockdown but not in a consistent manner across the group. The absence of socialising outside the home reduced anxiety in the children and had a positive effect on their GI symptoms, as did having unrestricted access to the toilet at home. On the contrary, restrictions on outdoor exercise and the cancellation of all sports clubs had a negative effect on the GI symptoms of some children. Changes in the child's usual routine caused by Covid-19 lockdowns and restrictions unsettled some children which prompted a worsening of GI symptoms. Overall, our results regarding the change in nature and severity of GI symptoms in autistic children during home confinement due to Covid-19 lockdown, reflects previous research which has indicated a complex interplay between GI symptoms and various aspects of behaviour, sleep and mood (including anxiety) [238] [97] [244] [58] [68] [69].

Finally, some lessons can be learned from the VIVO-ASD study to inform future intervention studies for GI symptoms in autistic children. Studies should be simple to understand and not involve too much of the parents' time. Involvement in the study should not cause the child to miss time at school. Parents need reassurance about possible side-effects of the interventions especially where it is a medication rather than a food supplement. Blood tests are not going to be acceptable to most families and stool or urine samples need to be collected at home. For appointments at the research centre, a quiet waiting area with good Wi-Fi is important. A taste test week at the beginning of the study allows parents to find out if their child will take the product and reduces the drop-out rate.

4.6 Key Lessons

In this study we sought to answer these questions;

1. What are the lived experiences of families managing persistent GI symptoms in their autistic child and what has been their experience of related healthcare?

2. What can we learn from the experience of participants in the VIVO-ASD clinical trial that could inform the design of future clinical trials to make them more accessible and acceptable to families with an autistic child with GI symptoms?

3. How has Covid-19 lockdown and Covid-safe measures in schools affected the child's GI symptoms and the family experience of managing these symptoms?

Key lessons from the findings for question 1;

- Parents are struggling with effective management of their autistic child's GI symptoms, and it is a source of stress for the parents and is impacting on the quality of life for the child and their family which increases at the child gets older

- For medical appointments autistic children need a quiet waiting room with good WIFI, advance information on the nature of the medical appointment and an approach to treatment that respects their sensory differences

- Clinicians should adjust how they communicate with autistic children in order to help them understand their GI condition, engage with the treatment plan and improve the chances of successful management of their GI condition

- Parents and autistic children may find it difficult to express the nature and severity of GI symptoms and attention should be given to changes in behaviour, sleep or eating habits that may be signs of GI distress
- Toilet training was problematic so families may benefit from specialist advice and resources that take account of the sensory differences in autistic children

Key lessons from the findings for question 2;

- Blood tests are not acceptable to most families of autistic children
- Stool or urine samples would have to be collected at home
- Participation in a study should not cause a child to miss school. This may mean assessments have to be done at the weekend, after school or during school holidays
- Participation should not be overly complex or time-consuming for parents
- Any treatment product the children are required to ingest should be bland tasting and a powder format is preferable to capsule or tablet. A taste test before joining the study can allay parent and child concerns about whether the child is able to take the product and improve participant retention in the study

Key lessons from the findings for question 3;

- Autistic children with social anxiety or anxiety about using school toilets may experience an improvement in their GI symptoms during home confinement

- Autistic children that need a lot of exercise and become anxious about any change in routine may experience a worsening of GI symptoms during home confinement

- Home confinement can offer an opportunity for parents to help autistic children improve their independent toileting skills or expand the range of foods they will eat

Chapter 5   Discussion

The objective of this research was to investigate the effectiveness of certain dietary interventions in improving both GI symptoms and global function in autistic children. I also sought to understand the impact GI symptoms have on autistic children and their families and to explore the experience of parents in managing their child's GI symptoms, including their experience of related healthcare. The interviews for the qualitative research were done during Covid-19 lockdown and so picked up information about how lockdown had affected the family experience of managing GI symptoms in their child.

The possible mechanisms of change for dietary intervention for autistic children with GI symptoms presented in Table 1-2 includes several actions that relate to the microbiota-gut-brain axis. In their recent review of the microbiota-gut-brain axis and its relevance to GI symptoms in autism [187] Hung and Gross Margolis state that many of the environmental and genetic factors that affect the development and function of the central and enteric nervous systems, are now being found to contribute to the development or symptom presentation of GI disorders in autistic children. Research has already shown that therapies manipulating the gut microbiota can show an improvement in GI symptoms and also behaviour in some autistic children [182][245]. We also know that GI symptoms in non-autistic children are associated with wider symptoms including sleep problems, anxiety, severe stress, long-term fatigue, headaches, nausea and feeling dizzy [52]. Hung and Gross Margolis [187] conclude that there is still much that is uncertain regarding the microbiome-gut-brain axis and GI disorders in autistic children, and also regarding how these might relate behaviour or other co-occurring conditions. As such, they recommend that treatment of GI disorders should remain conservative but consider the potential interplay with behaviour and other co-occurring conditions [187].

Diet is one of the major influences of the gut microbiome [263] and as such it is reasonable to expect that the personalised diet intervention reported in Chapter 2 could alter the gut microbiome. However, we have no proof of this except for the change in levels of two urine organic acids following the intervention and we only have this data for 18 participants. This is a real-world study using data routinely collected

during clinical practice and suffers from a number of limitations. At this point in time, research is very sparse regarding how changes in the ecology of the gut relate to improvements in health or otherwise [263]. Perhaps the most interesting part of the results of my personalised diet study was from the two responder analyses. These suggested that the action of the intervention was not simply to improve GI symptoms but was having a wider effect which aligns with current literature [264][265] and the mechanisms of change proposed in Chapter 1.

Considering the results of the Vivomixx probiotic study reported in Chapter 3, we did not find that Vivomixx showed more improvement in either GI symptoms or global wellbeing than the placebo. There are many possible reasons for this result which seems to contradict most other autism probiotic studies, and these are discussed in section 3.7. However, we did find a notable improvement in global wellbeing in around a third of the participants following Vivomixx treatment and further research with a more restricted subgroup may show positive efficacy for the whole group. Current probiotic research suggests that future autism probiotic studies may need to restrict the type of GI symptom and match this to a specific probiotic with evidence of success for this GI symptom. Research is pointing towards personalised probiotic interventions and, since an autism-specific gut microbiome pattern has not yet been identified, it is reasonable to expect that personalised probiotic interventions may be required in this patient group. This may need a pilot study to determine the stool microbiome analysis pattern that gives the best chance of response to the probiotic intervention. Alternatively, home testing for bowel transit time using muffins containing blue dye has proven to be a better predictor of the gut microbiome function than stool frequency or consistency, and this could be a simple and cheap way to differentiate a targeted population for a probiotic intervention [246]. We should also consider that recent autism probiotic studies [247][245] indicate that probiotics can benefit autistic children without GI symptoms, suggesting that the action of probiotics is not simply via reduction or resolution of GI distress. This reflects the possible mechanisms of change proposed in Chapter 1.

In the Vivomixx probiotic study we found a strong placebo effect in parent reports on their autistic child which resonates with existing literature [248]. In future probiotic studies with autistic children, it is imperative to have at least one biometric outcome

measure alongside parent, child and professional reports. We recognised this in the design of the VIVO-ASD study, but the stool sample collection and analysis were thwarted by Covid-19 lockdown. There are limitations on the possibilities for collection of biometric measures in this group and the results of my research on this (Chapter 4 section 4.3.3.1.1.) can offer some guidance for future studies on what families consider acceptable.

In the VIVO-ASD study parents completed paper questionnaires which were then scored, and these scores input into a spreadsheet. In our qualitative study, parents expressed a preference for regular or daily reporting via a simple phone app or via a weblink sent to them. More regular reporting can even out day-to-day variability in a child. Pertinent to future probiotic studies, Dieta Health have developed a smart phone app that is superior to patient self-report for Bristol Stool Type reporting in Irritable Bowel Syndrome (IBS), and is as accurate as the IBS gold standard of two expert physicians [249]. STRIPES is a web-based symptom tracking app that has been developed by Symplify LLC and there is a STRIPES version of a new autism-specific GI symptoms assessment, the Brief Parent-Report Screen for Common Gastrointestinal Disorders in ASD [250]. This is not yet commercially available but is being used in autism research in the USA and represents an accessible and convenient way of reporting for parents and allows the collection of large amounts of digital research data. These tools can help to improve compliance with reporting, allowing larger datasets to be collected and more reliable conclusions to be made.

Our qualitative research showed that autistic children's GI symptoms changed during home confinement with Covid-19 lockdown with a mixture of improvement and worsening in symptoms. This contrasts with most of the studies that have reported the effects of Covid-19 lockdowns on autistic children of varying ages, in a variety of countries. Some reported mostly negative effects [243] [255], while others have reported mostly positive effects [256] [257] [258] and finally a number have reported mixed effects [259] [260] [261] [262]. Few investigators reported on any aspect of self-care or dietary habits that may be relevant to the management of GI symptoms. Di Renzo et al. (2020) reported no change in self-care abilities (which included toileting) and no change in taste or smell sensitivity. Latzer et al. (2021) reported a mixed picture regarding dietary habits, with some parents reporting a worsening of

food-related behaviours (including food selectivity) while others reported greater flexibility in their child's dietary habits during lockdown. Meral (2022) found that parents reported an improvement in their child's ability to meet their own self-care needs, including performing a greater variety of self-care skills (toileting skills are specifically mentioned). Guidotti et al. (2020) reported that there was no change in children's appetite during lockdown. None of the studies have specifically focused on the management of GI symptoms in autistic children and access to related healthcare during the Covid-19 lockdowns and its restrictions.

Our mixed results regarding the change in nature and severity of GI symptoms in autistic children during home confinement due to Covid-19 lockdown, reflects previous research which has indicated a complex interplay between GI symptoms and various aspects of behaviour, sleep and mood (including anxiety) [238] [97] [244] [58] [68] [69]. The parents in our study reported that an increase in anxiety was a trigger for the onset of diarrhoea or for a worsening of constipation or reflux in their child. The prevalence of an anxiety disorder in autistic children has been estimated at 40% [94] and a lifetime prevalence of anxiety disorders in autistic adults is estimated at 42% [95]. Dovgan et al. (2022) found a bi-directional relationship between internalising symptoms like anxiety, and GI symptoms in autistic children [97] indicating that anxiety can affect GI symptoms and vice versa, and resolution of either may require the simultaneous treatment of both. In our study, parents generally reported a reduction in anxiety in their child due to the lack of social pressures while at home during Covid-19 lockdown, which may have contributed to the reported improvement in GI symptoms in some of our participants. This appears to contrast with Amorim et al. (2022) where parent-reported levels of anxiety in autistic children were significantly higher than those in not-autistic children during Covid-19 lockdown in Portugal. It should be noted that a non-standard measure of anxiety was used. [259]. Polonyiova et al. (2022) used a standard measure for internalising behaviour which included anxiety in a combined score with fear, sadness, apathy and social withdrawal [261]. They found significantly higher levels of parent-reported maladaptive internalising behaviour in autistic children compared to not-autistic children in Slovakia at all three timepoints studied (before lockdown, Wave 1 lockdown and Wave 2 lockdown). Comparing the mean scores for parent- assessed maladaptive internalising behaviour in autistic children from before lockdown to during Wave 2, there was a significant

increase in the level for autistic children. We did not measure anxiety levels in our participants as this was a qualitative study, but some parents reported an increase in anxiety in their child due to changes in their usual routine and this was accompanied by a worsening of their GI symptoms, which reflects the mechanisms of change proposed in Chapter 1.

Another result from our qualitative study is the finding of a lack of understanding and reasonable adjustments by NHS staff. The NHS has recognised autism and learning disabilities as a priority in their Long-Term plan and actions identified include increasing the understanding of the needs of autistic people amongst all NHS staff and reducing health inequalities for this group. The Oliver McGowen Mandatory Training in Learning Disability and Autism has completed a successful pilot within parts of the NHS and the final format of the training in under development. This training is included in the Health and Care Act 2022 which means that all staff registered with the Care and Quality Commission will complete this training. This should help staff throughout the NHS to communicate more successfully with autistic individuals and to be aware of, and adjust for, their sensory challenges in the healthcare setting and healthcare practice. Our results support the need for more awareness throughout the NHS about the communication and sensory needs of autistic children accessing healthcare, to reduce child and parent stress; to improve patient understanding of their condition and the treatment plan; and to remove barriers to accessing healthcare.

It is clear from our qualitative research that most parents are struggling to find the support they need to manage or resolve their autistic child's GI symptoms. Given the high prevalence of GI symptoms in autistic children [236], early screening for this following an ASD diagnosis may be a cost-effective option. There are currently no guidelines from professional gastroenterology bodies in the UK or USA for the assessment and treatment of GI distress specifically in autistic children. In non-autistic children managing chronic constipation and faecal incontinence is difficult due to the complex interplay between GI symptoms, psychological symptoms and environmental/social factors, combined with the lack of published evidence to guide clinical practice [64]. There is agreement that GI distress can present differently in autistic children [251]. Since conducting the Vivomixx study, two autism-specific

screening tools for GI distress have been developed in the USA, The Brief Parent-Report Screen for Common gastrointestinal Disorder in Autism Spectrum Disorder [250], and the ASD Gastrointestinal and Related Behaviours Inventory [55] but these are both awaiting clinical validation, and research on their suitability for the UK population is also needed. Further research is also needed on the identification and expression of pain by autistic children with GI symptoms and how they can be helped to recognise and express this. The Wong-Baker FACES pain rating scale [252] has been developed to help children aged as young as 3 years to communicate about their pain and there is evidence that this can prove a useful tool for some autistic children to recognise and communicate pain [253]. Early identification of autistic children with GI distress would allow parents to be provided with specialist education and resources to aid successful toilet training and management of GI symptoms in their child. Many resources are available on the E.R.I.C. charity website https://www.eric.org.uk/ but parents need to be signposted to this by their health professional, so they know it is trustworthy. Whether GI distress is present or not at the time of an autism diagnosis, parents and other carers should be made aware of the different presentation of GI symptoms and possible signs of GI distress in autistic children. This would enable them to seek medical help for their child in a timely fashion so that a bowel management plan can be developed or adjusted as the situation changes. There is a need for research to discover whether implementing earlier specialist support and guidance can reduce the incidence of chronic GI issues in this patient group and improve quality of life for the child and their family.

The stepped care model is a model already used in mental health and has been proposed for health and social care for autistic individuals [254]. It is a framework for organising healthcare and one of its objectives is to provide the right level of support at any stage and so avoid a healthcare crisis.   Regular monitoring allows the level of support to be changed as necessary. Considering the results of this study and relevant research, I have applied the Stepped Care model to services for autistic children with GI symptoms as an object for discussion.
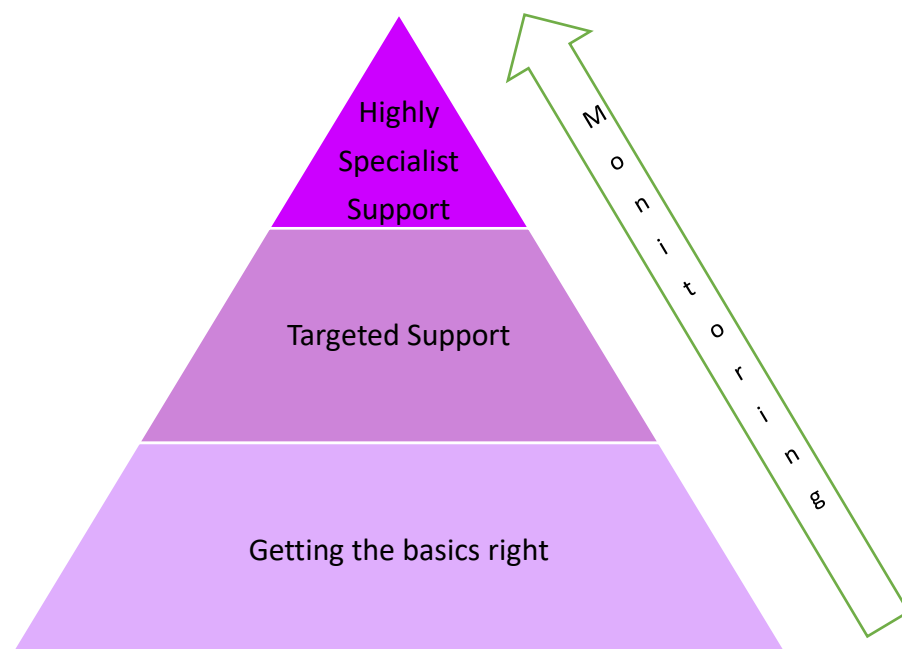
At the bottom of the triangle is the lowest level of support and could include; education for parents on sensory issues and toileting before beginning toilet training with their child; appropriate education for children on bowel and bladder health and function so they can understand this aspect of their body and improve communication of symptoms to their parents; pre-appointment information about medical appointments to reduce child and parent stress; a quiet waiting area; and a choice of in-clinic, video or phone consultations.

In the middle of the triangle is the next level of support which is targeted at particular GI issues and could include; setting treatment priorities with children and their parents; using autism specific tools to assess GI distress and monitor the effect of treatment; health services that adjust for anxiety, sensory and communication issues; and personalised treatment plans. This would also include the conservative treatment measures recommended by NICE such as laxatives, dis-impaction, stool analysis for infections and long-term follow-up.

At the top of the triangle is highly specialist support. This needs to embrace the complexity often presenting in autistic patients, particularly those with co-occurring conditions who may be taking several medications. It may require a multidisciplinary team to address the bi-directional relationship between GI issues and mental health

conditions such as anxiety: Athanasakos et al describe a multidisciplinary team approach which improved the child and parent severity rating for chronic constipation and fecal incontinence [64]. They used anorectal manometry for the assessment of sphincter function and rectal sensation, although this may not be possible for some autistic children due to sensory issues and the need for co-operation with instructions. However, there are many aspects of their bespoke treatment approach which target the physical, emotional and sociological aspects of childhood chronic constipation and fecal incontinence, that could inform practice with autistic patients. It is not clear from the published research by Athanasakos et al whether any of the participants were autistic. Although some gastroenterology investigative procedures may not be practical with this patient group, research shows that when necessary, endoscopies can be undertaken successfully with the right preparation and support for the individual and their parents [240]. More research is needed in the UK to identify the service adjustments needed for autistic children where an invasive GI investigative procedure is required, and to establish best practice guidelines for assessment and treatment of GI symptoms in autistic children. In the USA this need has led to the establishment of a Consortium for Autism, Neurodevelopmental Disorders and Digestive diseases (CANDID) which is a multi-disciplinary organisation involving clinicians, researchers, and parent-led organisations. CANDID aims to understand the problems experienced by parents of children diagnosed with neurodevelopmental disorders (NDDs) including autism, in managing their child's GI issues, and to promote research and clinical education to improve assessment and treatment of GI issues (more can be found out about CANDID at https://www.candidgi.com/about-candid/).

The NHS England 5-year autism research strategy states that more evidence needs to be generated and assessed on healthcare treatments and policies for autistic individuals to improve the health outcomes of this patient group. The strategy identifies a number of steps needed to improve autism healthcare research including the establishment of a national register of autistic individuals who are interested in receiving invitations to participate in clinical research. The difficulties in recruitment to the VIVO-ASD study supports the need for such a register in England. An example of the success of such an approach is the Autism Speaks Autism Treatment Network (ATN) which has been established in the USA to develop effective medical care specifically for autistic children and adolescents. It is a network of clinicians,

researchers, and families at 17 locations around the USA. This network has facilitated several successful multi-centre research studies on GI problems in autistic children, diet interventions and the role of the gut microbiome and metabolome. There is a need for a similar network to be set up in the UK to progress research and generate evidence for effective assessment and treatment of GI symptoms in autistic children. One of the strengths of the VIVO-ASD probiotic study was that the study group was very mixed and included many participants with additional challenges such as learning disability and being minimally-verbal. Despite this, we were able to achieve good adherence for the group as a whole. This demonstrates the feasibility of intervention studies which include often overlooked groups within the autism spectrum and should encourage researchers to include autistic children with extra challenges.

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Appendices

Autism Treatment Evaluation Checklist

PARENT QUESTIONNAIRE

Please complete this questionnaire on behalf of your child and give to the researcher.

Today's date (DD/MM/YY):_____Child's participant ID:_____

In this section you will complete an ATEC evaluation of your child's overall function. ***You will be asked to complete the ATEC evaluation 3 times during the course of this trial and it is important that the same person completes it each time.***

Autism Treatment Evaluation Checklist (ATEC)
Bernard Rimland PhD and Stephen M Edelson PhD, Autism Research Institute, 4182 Adams Avenue, San Diego, CA 92116, www.autism.com/ari
This form is intended to measure the effects of treatment.

Your relationship to child:_____

1. Speech, language and communication
Tick to indicate whether the statements are NOT true, SOMEWHAT true or VERY true about your child;

	ATTRIBUTE	Not true	Somewhat true	Very true
1	Knows own name			
2	Responds to 'No' or 'Stop'			
3	Can follow some commands			
4	Can use one word at a time (No!, Eat, Water, etc)			
5	Can use 2 words at a time (Don't want, Go home)			
6	Can use 3 words at a time (Want more milk)			
7	Knows 10 or more words			
8	Can use sentences with 4 or more words			
9	Explains what he/she wants			
10	Asks meaningful questions			
11	Speech tends to be meaningful/relevant			
12	Often uses several successive sentences			
13	Carries on a fairly good conversation			
14	Has normal ability to communicate for his/her age			

2. Sociability

Tick to indicate whether the statements are NOT true, SOMEWHAT true or VERY true about your child;

	ATTRIBUTE	Not true	Somewhat true	Very true
1	Seems to be in a shell – you cannot reach him/her			
2	Ignores other people			
3	Pays little or no attention when addressed			
4	Uncooperative and resistant			
5	No eye contact			
6	Prefers to be left alone			
7	Shows no affection			
8	Fails to greet parents			
90	Avoids contact with others			
10	Does no imitate			
11	Dislikes being held or cuddled			
12	Does not share or show			
13	Does not wave "bye bye"			
14	Disagreeable /not compliant			
15	Temper tantrums			
16	Lacks friends/companions			
17	Rarely smiles			
18	Insensitive to other's feelings			
19	Indifferent to being liked			
20	Indifferent if parent(s) leave			

3. Sensory/Cognitive Awareness

Tick to indicate whether the statements are NOT true, SOMEWHAT true or VERY true about your child;

	ATTRIBUTE	Not true	Somewhat true	Very true
1	Responds to own name			
2	Responds to praise			
3	Looks at people and animals			
4	Looks at pictures (and TV)			
5	Does drawing, colouring or art			
6	Plays with toys appropriately			
7	Appropriate facial expression			
8	Understands stories on TV			
9	Understands explanations			
10	Aware of environment			
11	Aware of danger			
12	Shows imagination			
13	Initiates activities			
14	Dresses self			

15	Curious, interested				
16	Venturesome – explores				
17	"Tuned in" – not spacey				
18	Looks where others are looking				

4. Health/Physical/Behaviour

Tick to indicate whether the statements are NOT a problem, a MINOR problem, a MODERATE problem or a SERIOUS problem with your child;

	ISSUE	NOT a problem	MINOR problem	MODERATE problem	SERIOUS problem
1	Bedwetting				
2	Wets pants/nappies				
3	Soils pants/nappies				
4	Diarrhoea				
5	Constipation				
6	Sleep problems				
7	Eats too much/too little				
8	Extremely limited diet				
9	Hyperactive				
10	Lethargic				
11	Hits or injures self				
12	Hits or injures others				
13	Destructive				
14	Sound sensitive				
15	Anxious/fearful				
16	Unhappy/crying				
17	Seizures				
18	Obsessive speech				
19	Rigid routines				
20	Shouts or screams				
21	Demands sameness				
22	Often agitated				
23	Not sensitive to pain				
24	"Hooked" or fixated on certain objects/topics				
25	Repetitive movements (stimming, rocking etc)				

Buie assessment

Score: 0 = None, 1 = Mild, rare (monthly), 2= Moderate, occasional (weekly), 3 = Severe, frequently (daily)

Vocal Behaviours		Motor Behaviours		Changes in Overall State	
Frequent clearing of throat, swallowing, tics etc.		Facial Grimacing		Sleep disturbances: difficulties getting to sleep, difficulties staying asleep	
Screaming		Gritting teeth		Increased irritability(exaggerated responses to stimulation)	
Sobbing "for no reason at all"		Wincing		Non-compliance with demands that typically elicit an appropriate response (oppositional behaviour)	
Sighing, whining		Constant eating/ drinking/ swallowing/ ("grazing" behaviour)			
Moaning, groaning		Mouthing behaviours: chewing on clothes (shirt sleeve cuff, neck of shirt etc), pica (eating non-food items))			
Delayed echolalia that includes references to pain or stomach (e.g. child says, "Does your tummy hurt?" echoing what mother may have said to child in the past)		Application of pressure to the abdomen: leaning abdomen against or over furniture or kitchen sink, pressing hand into abdomen, rubbing abdomen			
Direct verbalisations (e.g. child says "tummy hurts" or says "ouch", "owe", "hurts", or "bad" while pointing to abdomen)		Tapping behaviour: finger tapping on throat			
		Any unusual posturing , which may appear as individual postures or in various combinations: jaw thrust, neck torsion, arching of back, odd arm positioning, rotational distortions of trunk/torso, sensitivity to being touched in abdominal area, flinching			
		Agitation: pacing, jumping up and down			
		Unexplained increase in repetitive behaviours			
		Self injurious behaviours: biting, hits/slaps face, head-banging, unexplained increase in self-injury			
		Aggression: onset of, or increase in, aggressive behaviour			

Total Score: Intensity = total/ no. of non-zero scores   Ranges   0-10   11-20   21-30   30+