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Protocol for the Gut Bugs in Autism Trial: a double-blind randomised placebo-controlled trial of faecal microbiome transfer for the treatment of gastrointestinal symptoms in autistic adolescents and adults

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ABSTRACT

Introduction Autism (formally autism spectrum disorder) encompasses a group of complex neurodevelopmental conditions, characterised by differences in communication and social interactions. Co-occurring chronic gastrointestinal symptoms are common among autistic individuals and can adversely affect their quality of life. This study aims to evaluate the efficacy of oral encapsulated faecal microbiome transfer (FMT) in improving gastrointestinal symptoms and well-being among autistic adolescents and adults.

Methods and analysis This double-blind, randomised, placebo-controlled trial will recruit 100 autistic adolescents and adults aged 16–45 years, who have mild to severe gastrointestinal symptoms (Gastrointestinal Symptoms Rating Scale (GSRS) score ≥2.0). We will also recruit eight healthy donors aged 18–32 years, who will undergo extensive clinical screening. Recipients will be randomised 1:1 to receive FMT or placebo, stratified by biological sex. Capsules will be administered over two consecutive days following an overnight bowel cleanse with follow-up assessments at 6, 12 and 26 weeks post-treatment. The primary outcome is GSRS score at 6 weeks. Other assessments include anthropometry, body composition, hair cortisol concentration, gut microbiome profile, urine/plasma gut-derived metabolites, plasma markers of gut inflammation/permeability and questionnaires on general well-being, sleep quality, physical activity, food diversity and treatment tolerability. Adverse events will be recorded and reviewed by an independent data monitoring committee.

Ethics and dissemination Ethics approval for the study was granted by the Central Health and Disability Ethics Committee on 24 August 2021 (reference number: 21/CEN/211). Results will be published in peer-reviewed journals and presented to both scientific and consumer group audiences.

Trial registration number ACTRN12622000015741.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the largest registered randomised clinical trial of fecal microbiome transfer in autistic adolescents and adults codesigned in consultation with autism advisors and autism support organisations.
⇒ The double-blind, placebo-controlled design and use of capsules as a non-invasive method of delivery are the main strengths of this clinical trial.
⇒ Conducting a 6-month follow-up after a single treatment with fecal microbiome will allow identification of a possible lag between treatment and changes in gastrointestinal (GI) symptoms.
⇒ Our study will focus on autistic individuals, so the findings may not extrapolate to other individuals with GI symptoms.
⇒ Faecal microbiome transfer is not a standardised treatment paradigm, and therefore, can result in varied responses due to donor differences, recipient factors and procedural variations.

INTRODUCTION

Autism encompasses a group of complex neurodevelopmental conditions,[1] and co-occurring chronic gastrointestinal (GI) symptoms are common among autistic individuals.[2 3] These functional GI symptoms have been reported to be more common among those with higher and more complex support needs, adversely affecting their quality of life.[4]

Worldwide, approximately 1 in 50 people are diagnosed with autism, with males four times more likely to be diagnosed than females.[2] In New Zealand (NZ), it is estimated that more than 90 000 individuals may be autistic.[5–8] The precise neurological differences in autism likely involve a complex

interplay of genetic and environmental factors, with the gut microbiome potentially playing an important role.[2 9] More than 500 genes may be involved in autism,[10] some of which may also be key developmental regulators of both the central nervous system (CNS) and GI system.[10] Furthermore, there is increasing evidence that the gut–brain axis (whereby bidirectional signalling occurs between the GI system and the CNS) is mediated, at least in part, by the gut microbiome.[11] Additional factors have been associated with autism include gut microbial dysbiosis, nutritional deficiencies, immune system dysfunction and allergies.[10 12]

The gut microbiome plays many complex roles in the maintenance of health and protection against diseases.[13] These include provision of essential nutrients, prevention against proliferation of pathogenic micro-organisms, production of metabolites with anti-inflammatory and antimicrobial properties, and maturation of the host's immune system.[14–16] This symbiotic relationship between the gut microbiome and the human host is essential, and microbial dysbiosis has been observed across a range of metabolic, immune and neurobehavioural conditions.[13] Gut dysbiosis has been suggested to contribute to the neurological challenges and lower quality of life that accompanies GI symptoms seen in autism.[17] The gut microbiome of autistic individuals is characterised by lower bacterial diversity and relative abundances of potentially beneficial bacterial taxa compared with non-autistic individuals.[18–20] Alterations in the gut microbial composition have been associated with an increased inflammatory response, due to the disruption of the gut mucosal barrier, and increased intestinal permeability to gut metabolites and exogenous dietary peptides.[21] Further, it is also associated with disruptions of the neural, endocrine and metabolic signalling of the gut–brain axis.[21] Together, these mechanisms may contribute to some of the reduced quality of life experienced by autistic individuals.[17 21]

No pharmaceutical agents have been approved to treat the chronic GI problems experienced by autistic people.[2 22] However, there is an increasing interest in the potential of microbiota-based therapeutics for gut issues in autistic people, with clinical studies looking at the use of antibiotics, prebiotics, probiotics and faecal microbiome transfer (FMT) to ameliorate gut dysbiosis and reduce GI distress.[23–26]

Broad-spectrum antibiotics result in only temporary improvements in GI symptoms in autistic children, suggesting that antibiotics alone are unable to reverse gut microbial dysbiosis and maintain improvements in the long term.[24] Further, prebiotics and probiotics have shown limited efficacy in reducing GI symptoms and distress.[25] FMT has been postulated to be effective in alleviating GI symptoms in autistic individuals and may be a more effective treatment paradigm than probiotics.[4] In contrast to probiotics which contain one to several bacterial species, FMT contains not only hundreds but also viruses and fungi from the GI tract.[27]

Autistic children given cultured intestinal bacteria daily for 3 months were reported to have improvements in GI symptoms and distress.[28] Daily FMT treatment over 8 weeks in autistic children showed similar improvements.[29] Long-term improvements in gut and behavioural symptoms up to several years post-FMT have also been demonstrated in several studies, with sustained changes in the gut microbiome composition.[29–31] Although these findings are promising, these studies have limitations that require clarification before FMT can be considered as a treatment option for GI problems in autistic individuals. These limitations include small number of participants, open-label designs without a control group, demanding treatment protocols including antibiotics and prolonged FMT treatment over several months, as well as the use of proton-pump inhibitors for the duration of FMT treatment. FMT delivered with such intensive treatment protocols is unlikely to be a feasible therapy that would be readily acceptable by patients in clinical settings. A larger more robust randomised placebo-controlled trial using a potentially more acceptable and less demanding protocol with encapsulated FMT is needed, to ascertain whether FMT is an effective and applicable therapy for GI issues in autism.

AIM

To evaluate the efficacy of oral encapsulated FMT in improving GI symptoms and well-being among autistic adolescents and adults. The study will be conducted at the Liggins Institute's Clinical Research Unit (University of Auckland), in Auckland, NZ.

METHODS

Study design

This study is a two-arm, double-blind, placebo-controlled randomised clinical trial with autistic individuals randomly assigned to either treatment (encapsulated gut microbiome) or placebo (encapsulated saline solution), stratified by biological sex at birth. Eligible participants will be followed for 26 weeks after randomisation (see figure 1).

Participant recruitment and eligibility

Recipients

We aim to recruit 100 autistic adolescents and adults aged 16–45 years of age, who must meet the inclusion and exclusion criteria outlined in table 1. The decision to use a GSRS of >=2 as an inclusion crteria is based on a previous FMT study in Autism[29] in which the average symptom burden was scored as >2 at the start of the trial and resolved to <2 post-treatment.

Donors

We aim to recruit eight stool donors, with participants receiving faecal microbiome from four donors. This is made up of four primary and four reserve donors if any

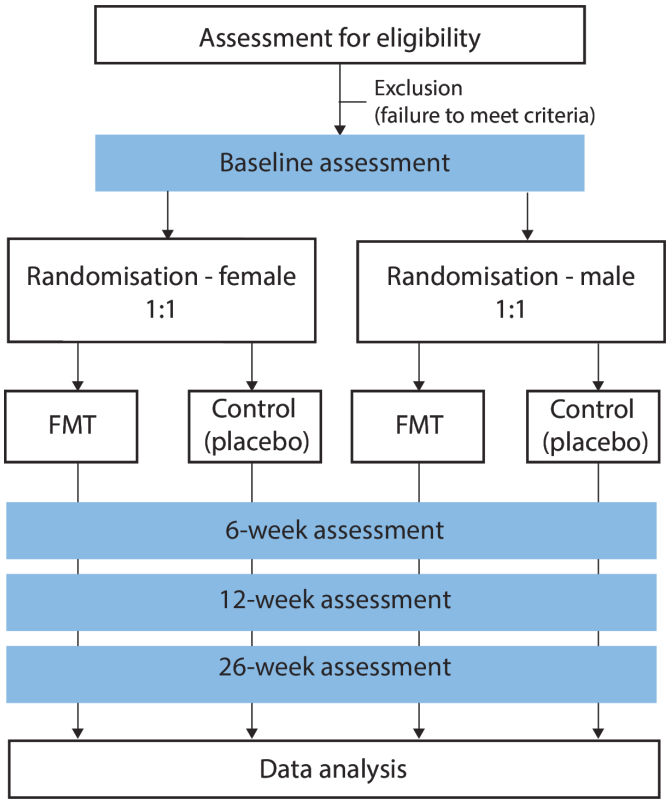

Figure 1 Diagram showing flow of participants (recipients) in the Gut Bugs in Autism Trial randomised to faecal microbiome transfer (FMT) or placebo (control).

of the primary donors are unwell/unavailable during the treatment process. The same four primary donors will be asked to donate regularly throughout the trial to minimise treatment heterogeneity. The donor inclusion and exclusion criteria are listed in table 2. To eliminate the risks of pathogen transmission, screening equivalent to those used for blood donation in NZ will be performed.[32] Donors will also be screened for a range of human pathogens, using antigen, antibody, culture, microscopy and DN-based methods including the BioFire FilmArray Gastrointestinal PCR Panel (table 3).

Donors will be asked to visit the research site as required and at least every 6 months to provide fresh stool samples for capsule production. Pathogen screening will be repeated for every capsule batch. If multiple stool samples from the same donor are required for one batch of capsules, repeat screening will only be performed if two or more weeks have elapsed since their last pathogen screen. We will interview potential donors to gather information about high-risk behaviours or activities that would exclude them from the trial. As irritable bowel syndrome (IBS) may be related to the gut microbiome, we will exclude potential donors who may have IBS, using a modification of the Rome criteria, whereby a positive screen is defined as having ≥3 episodes of abdominal pain per month as described in part 1 of the criteria, as well as an additional symptom as defined in part II.[33] This rigorous screening process will also be carried out prior to every stool donation as per previous studies.[34 35] If a pathogen is detected, capsules from that donor will not be used and the donor stood down.

Donor stools will be processed as described previously to enrich the microbial component which will be doubly encapsulated and administered orally to recipients.[35 36] Briefly, we will use the plant-based DRcaps capsules (Capsugel, Sydney, New South Wales, Australia) that mask taste, odour and visual appearance, and are acid-resistant remaining intact during passage through the stomach,[37–39] ensuring FMT delivery to the proximal bowel.[37] Prepared FMT capsules will be stored at −80°C and remain viable for up to 6 months.[35] We previously

Table 1 Inclusion and exclusion criteria for participants (recipients) who will undergo faecal microbiome transfer

Inclusion	Aged 14–45 years at recruitment
	Previous formal diagnosis of autism (including ASD, Asperger's syndrome, autistic disorder and PDD-NOS) as characterised in DSM-4, DSM-5 or ICD-10-AM
	Mild to severe gastrointestinal symptoms with mean overall GSRS score ≥2.0
	Ability to swallow treatment capsules
	Willing to complete the clinical assessments at baseline and follow-up visits
Exclusion	Oral or parenteral antibiotic use within the preceding month
	Intake of probiotic supplements within the preceding month
	Regular oral steroid treatment
	Dependence on enteral tube feeding
	Serious medical problems that require specific treatment
	Moderate to severe depression and/or suicidal ideation as per PHQ-9
	Pregnancy or planned pregnancy
	Known allergy to any medications or foods and/or to macrogol

ASD, autism spectrum disorder; DSM, Diagnostic and Statistical Manual of Mental Disorders; GSRS, Gastrointestinal Symptoms Rating Scale; ICD-10-AM, International Statistical Classification of Diseases and Related Health Problems; PDD-NOS, Pervasive Developmental Disorder-Not Otherwise Specified; PHQ-9, Patient Health Questionnaire-9.

Table 2 Inclusion and exclusion criteria for stool donors

Inclusion	Aged 18–32 years at recruitment
	BMI 18.5–30.0 kg/m^2
	Total body fat ≤33% for females and ≤22% for males based on DXA scans
	Healthy diet (ie, consumption of ≥4 portions of fruit and/or vegetables per day)
	Regular exercise (ie, moderate to vigorous physical activity for ≥3.5 hours per week)
	Regular bowel habit (ie, at least once every 2 days)
Exclusion	Any transmissible viral, bacterial or protozoan pathogens including multidrug-resistant organisms*
	Gastrointestinal disease (eg, IBD, IBS, coeliac disease or eosinophilic oesophagitis)
	Metabolic conditions (eg, pre-diabetes, diabetes, metabolic syndrome, hypertension or dyslipidaemia)
	Impaired fasting glucose (plasma glucose >5.9 mmol/L) or HbA1c>41 mmol/mol
	Atopic diseases requiring regular high dose oral of inhaled steroid prophylaxis or treatment (eg, asthma or eczema)
	Previous formal diagnosis of autism (including ASD, Asperger's syndrome, autistic disorder and PDD-NOS) as characterised in DSM-4, DSM-5 or ICD-10-AM
	Previous diagnosis of mental health issues, including eating disorders
	Diagnosed genetic/syndrome disorders with health sequelae
	Current or history of malignancy
	Use of oral antibiotics or probiotics in the last 3 months
	Regular binge drinking (>5 standard alcoholic units/session at least once a week)
	Any recent use of recreational drugs, tobacco or vaping
	Current or past pregnancy
	Overseas travel in the past 6 months with high risk of gastrointestinal infections†

*Pathogen screening will be repeated for every capsule batch.
†This criterion is to exclude any potential donor who may acquire any viral, bacterial or intestinal infections during their overseas travel. Donors returning from travel abroad will need to wait a minimum period of 2 weeks from their arrival back in New Zealand before donating and postdiscussion with trial clinician.
BMI, body mass index; DSM, Diagnostic and Statistical Manual of Mental Disorder; DXA, whole-body dual-energy X-ray absorptiometry; HbA1c, glycated haemoglobin; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; ICD-10-AM, International Statistical Classification of Diseases and Related Health Problems.

observed no major adverse events recorded in association with this treatment.[36]

Randomisation procedure

Participants will be randomised in a 1:1 ratio to either FMT or placebo groups, stratified by biological sex, using block randomisation with variable block sizes of 2 and 4r. Since males are four times more likely to be diagnosed with autism than females,[2] all eligible participants, irrespective of sex, will be randomised into the trial to maximise the likelihood of reaching our target sample size. Thus, we aim to achieve a 1:1 ratio between randomisation groups within each sex but not between sexes.

An investigator not involved with the recruitment, treatment or assessment of participants will perform the group allocation in three steps:

1. Allocate the participants to group A or B according to a computer-generated randomisation sequence.[40]
2. Obtain the packs containing the intervention capsules (independently labelled A or B by a technician).
3. Allocate the packs to participants according to their unique code.

Note that both researchers and participants will be blinded to capsule contents throughout the trial.

Intervention

All participants will undergo partial bowel cleansing the afternoon prior to treatment using an oral solution containing 70 g macrogol 3350 (polyethylene glycol) (Glycoprep Orange, Fresenius Kabi, Auckland, NZ). This has been demonstrated to be efficacious at reducing bacterial diversity and microbial population.[35 41 42] This will be followed by the intervention, delivered as 20 capsules taken over 1–2 days (20 once or 10 per day). In total, FMT recipients will receive five capsules from each of the four donors. The total FMT dose corresponds to 1o gm of concentrated microbiota. Placebo recipients will receive 20 visually identical capsules containing saline with 15% glycerol and 10 mg/mL cocoa. Treatment will be given in the clinic and supervised by members of the research team. Participants will remain fasted post the bowel cleansing for >= 8 hours before taking the capsules the following morning. After the treatment, participants will be asked to remain fasted for another 2 hours to help

Table 3 Pathogen screen for donors

Sample	Viruses	Bacteria	Parasites
Blood	Hepatitis A, B and C	*Treponema pallidum* (syphilis)	*Strongyloides* spp*
	HIV		
Stool	Human adenovirus F40/41	*Campylobacter* spp	*Cryptosporidium* spp
	Astrovirus spp	*Clostridioides difficile* toxin A/B	*Cyclospora cayetanensis*
	Norovirus GI or GII	Diarrheagenic *Escherichia coli*/*Shigella* spp:	*Entamoeba histolytica*
	Rotavirus A	► Enteroaggregative *Escherichia coli*	*Giardia lamblia*
	Sapovirus spp	► Enteroinvasive *E. coli*	Any ova, cysts or parasites†
		► Enteropathogenic *E. coli*	
		► Enterotoxigenic *E. coli*	
		► Shiga-like-toxin-producing *E. coli*	
		Helicobacter pylori	
		Multidrug-resistant organisms:	
		► Carbapenem-resistant organisms	
		► ESBL-producing Enterobacteriaceae	
		► Vancomycin-resistant *Enterococcus* spp	
		Plesiomonas shigelloides	
		Salmonella spp	
		Vibrio spp	
		Yersinia enterocolitica	
Nasal	SARS-CoV-2	–	–

*Only performed if the donor has a history of travel to the tropics.
†On microscopic examination.
ESBL, extended spectrum beta-lactamase.

minimise the length of time the capsules will remain in the stomach.

Outcomes

Primary outcome

- GSRS[43] score at 6 weeks.

Secondary outcomes

- GSRS score at 12 and 26 weeks.
- Well-being and behaviour from the following questionnaires at 6, 12 and 26 weeks:
  Patient Health Questionnaire-9 (PHQ-9).[44]
  Generalised Anxiety Disorder Screener.[45]
  Short Warwick-Edinburgh Mental Well-being Scale.[46–48]
  Perceived Stress Scale.[48]
  Physical activity (International Physical Activity Questionnaire-Short Form[49] Sleep quality assessed with the Sleep Quality Scale[50] Food selectivity and diversity analysis using the NZ Adolescent Food Frequency Questionnaire).[50]
- Gut microbiome profile at 6, 12 and 26 weeks:
  Engraftment of donor gut microbiome.
  Recipient gut microbiome composition and diversity
  Bacteriophage diversity.
- Body mass index and SD score, waist-to-hip ratio and waist-to-height ratio at 6, 12 and 26 weeks.
- Body composition (percentage of fat mass and fat-free mass) from dual-energy X-ray absorptiometry scan at 26 weeks.
- Hair cortisol levels at 6, 12 and 26 weeks.
- Urine and plasma gut-derived metabolite profiles at 6, 12 and 26 weeks.
- GI inflammation and permeability at 6, 12 and 26 weeks.

Safety monitoring

By adopting strict selection criteria for donors, we will minimise the potential transmission of pathogenic organisms following FMT. Participants will take each dose of FMT in our clinic under the supervision of a research clinician and/or nurse, where they will remain under close monitoring for at least 1 hour afterwards. Based on our previous experience and existing evidence, it is unlikely that participants will experience any severe adverse events.[35] However, participants will be instructed to seek immediate medical attention if they develop any severe adverse reactions following treatment. We will contact participants 24 hours after ingestion of each set of capsules, as well as 1, 3, 6, 12 and 26 weeks after treatment to enquire about any adverse side effects (see online supplemental material 1). Specifically, participants

will be asked to report on the following events: any changes in bowel movements, loose or bloody stools, abdominal pain, vomiting or nausea, fever, excessive burps, malodorous burps or any other symptom they might experience that could be related to treatment.

For PHQ-9, if the participant answers 'several days', 'more than half the days', or 'nearly every day' to question nine on suicidal ideation ('Thoughts that you would be better off dead or of hurting yourself in some way') one of the research clinicians in consultation with the research team psychiatrist will interview the participant further. Clinical follow-up will be recommended to the participant and the participant's consent sought to alert the participant's routine care provider/general practitioner (GP) and/or the research team psychiatrist will be updated to ensure additional mental health support is provided. Any adverse event data will be reviewed by an independent data monitoring committee (DMC) who can decide to stop the trial if the safety of participants is thought to have been compromised. The DMC will be notified within 48 hours of any serious adverse events and relevant clinical information provided.

Questionnaires

All questionnaires will be completed by the participants themselves online using data capture tools from the web-based research platform REDCap (Research Electronic Data Capture), hosted on secure servers at the University of Auckland. At study entry, we will collect demographic and medical information from participants including their age, sex assigned at birth, gender identity, self-reported ethnicity,[51] socioeconomic status,[52] age when first diagnosed as being autistic, and any current use of medications. Ritvo Autism Asperger Diagnostic Scale-Revised[53] will be used to charcterise the study population in terms of language, social relatedness, sensorimotor and circumscribed interests. Participants will also be asked to complete a range of validated health questionnaires at baseline, 6, 12 and 26 weeks treatment (see table 4). After their final dose of capsules, participants will complete a short questionnaire to gather their views and experience of taking the treatment (see online supplemental material 2). The success of treatment blinding will be assessed at the 6-week visit using Bang's Blinding index[54] (see online supplemental material 3).

Table 4 Timing of the various assessments that will be performed in this study

		Baseline	24 hours	48 hours	1 week	3 weeks	6 weeks	12 weeks	26 weeks
Auxology	Anthropometry	✓					✓	✓	✓
Body composition	DXA scans	✓							✓
Questionnaires	Demography	✓							
	RAADS-R	✓							
	General health and well-being	✓					✓	✓	✓
	NZAFFQ	✓					✓	✓	✓
	GAD-7	✓					✓	✓	✓
	GSRS	✓					✓	✓	✓
	IPAQ-SF	✓					✓	✓	✓
	PHQ-9	✓					✓	✓	✓
	PSS-10	✓					✓	✓	✓
	SQS	✓					✓	✓	✓
	WEMWBS-S	✓					✓	✓	✓
Tissue samples	Hair cortisol	✓					✓	✓	✓
	Stool markers	✓					✓	✓	✓
	Stool metagenomics	✓					✓	✓	✓
	Urine metabolites	✓					✓	✓	✓
	Plasma metabolites	✓					✓	✓	✓
Other	Adverse events		✓	✓	✓	✓	✓	✓	✓
	Tolerability of treatment			✓					
	Bang's Blinding Index						✓		

DXA, whole-body dual energy X-ray absorptiometry; GAD-7, General Anxiety Disorder 7-item; GSRS, Gastrointestinal Symptoms Rating Scale; IPAQ-SF, International Physical Activity Questionnaire-Short Form; NZAFFQ, New Zealand Adolescent Food Frequency Questionnaire; PHQ-9, Patient Health Questionnaire-9; PSS-10, Perceived Stress Scale 10-item; RAADS-R, Ritvo Autism Asperger Diagnostic Scale-Revised; SQS, Sleep Quality Scale; WEMWBS-S, Warwick–Edinburgh Mental Well-being Scale-short version.

Stool sample collection

Stool samples will be collected from participants at baseline (prior to bowel cleanse), and 6, 12 and 26 weeks after treatment to assess potential changes in the gut microbiome, metabolome and levels of intestinal inflammation and permeability by measuring faecal markers including calprotectin, lactoferrin, M2-pyruvate kinase, S100A12 and zonulin.[55 56] Where possible, stool samples will be collected on-site. If the sample is collected at home, participants will be asked to store their samples frozen. On receipt, stool samples will be aliquoted and stored at −80°C. The stool collected in the DNA/RNA Shield Faecal Collection tube (Zymo Research International, USA) will be reserved for gut microbiome assessment. The stool collected in the specimen pottle will be reserved for metabolomics and intestinal inflammation.[57]

Gut microbiome profiling

DNA and RNA will be extracted as previously described.[34 35] Shotgun metagenomic and metatranscriptomic sequencing will be performed by a commercial provider using Illumina's paired-end sequencing technology. Sequencing data will be processed as performed previously,[34] using the most recent bioBakery tools for meta-omic profiling.[34] We will use single nucleotide polyorphism (SNP) haplotypes to compare the genetic similarity of donor and recipient strains before and after treatment to assess the proportion and stability of donor strain engraftment.

Urine and blood metabolomic profiling

Participants will be asked to collect urine into a sterile container and this will then be stored at −80 °C until analysis. Blood samples will be collected via venepuncture from non-fasting participants in plain tubes, centrifuged and serum collected into cryovials and stored at −80 °C until analysis. Sample processing and metabolomic identification and quantitation will be performed via liquid chromatography with tandem mass spectroscopy (LC-MS/MS) as per previously published studies.[23 58]

Hair Cortisol profiling

Hair sample collection: Approximately 50–100 hairs will be collected from the posterior vertex as close as possible to the scalp and stored at −80 °C until analysis with LC-MS.

Sample size calculation

Our power calculation was based on a mean and SD of 2.44 and 0.47, respectively, for GSRS overall scores at baseline among 18 children and adolescents (aged 7–16 years) diagnosed as autistic.[29] We aim to recruit 50 participants per group, which will allow us to detect a statistically significant difference in GSRS overall scores of 0.31 (≈12.7%) between groups, based on a two-tailed t-test with 90% power and α=0.05. In the event of a 20% loss to follow-up, with 40 participants per group, our study would still be powered to detect a statistically significant difference in GSRS overall scores of 0.35 (≈14%) between groups.

Statistical analysis plan

Demographic and clinical characteristics of the study population at baseline will be summarised by randomisation group. Treatment efficacy for the primary outcome (ie, GSRS at 6 weeks) will be assessed on the principle of intention to treat, using data collected from all randomised recipients. Data will be analysed using a general linear model adjusting for the GSRS value at baseline and sex (stratification factor), with the adjusted mean difference and respective 95% CI reported. The interaction between randomisation group and sex will be tested, and sex-specific differences reported as appropriate.

Data on secondary outcomes will be analysed using random effects mixed models based on repeated measures, including the study ID as a random factor to account for the non-independence of measurements on the same participant. Models will include as independent variables randomisation group, study visit, their interaction term (group×visit), the baseline value of the outcome and sex.

Potential sex-specific treatment effects will be assessed on all outcomes by including an interaction term between sex and randomisation group in the main models. Nonetheless, irrespective of the detection or not of a statistically significant group×sex interaction, separate exploratory analyses by sex will also be conducted.

For categorical secondary outcomes (eg, adverse events), rates will be compared between randomisation groups using Fisher's exact tests, with their likelihood examined using multivariable generalised linear regression models based on a Poisson distribution. Effect sizes will be expressed as the adjusted relative risks (aRR) and respective 95% CI. If a relatively rare outcome has an unbalanced occurrence, the aRR may be derived using the modified Haldane-Ascombe method,[59] with 0.5 added to all values for a given outcome (which will be initially coded as 1 for an event and 0 for none).

Per-protocol analyses may be carried out on the primary and secondary outcomes excluding recipients with major protocol violations (eg, being given a course of antibiotics soon after treatment).

Data analyses will likely be performed by using SAS V.9.4 (SAS Institute) and SPSS V.29 (IBM). Missing data on the primary outcome will not be imputed. All statistical tests will be two sided at $p<0.05$. Given the exploratory nature of our secondary outcomes, we will not adjust for multiple comparisons, favouring a cautious interpretation of results to mitigate the risk of inflated type II errors due to conservative automatic p value adjustments.[60–64] Trial findings will be reported according to CONSORT (consolidated standards of reporting trials) 2010 guidelines.[65] The SPIRIT (standard protocol items: recommendations for intervention trials) reporting was used for this submission.[66]

Study status

The recruitment of recipients for the trial began in February 2023. It is expected that study recruitment will be completed in late 2024.

Patient and public involvement

Our study was developed via a 'codesign approach' with invaluable support and input from Autism NZ, their community advisory group and Altogether Autism, which are key organisations supporting autistic people in NZ. We have had a successful engagement with the chief executive and science leader of Autism NZ, and one of the investigators (RM) is a member of their community advisory group. RM has offered critical insight into the views, expectations and potential issues for participants and their families. We have also engaged with Altogether Autism through their national manager, who, together with their consumer and professional advisory groups, support the study. Further, critical input has also been provided by Keri Opai (Tātāriki Cultural Lead at Wise Group) and the Liggins Institute's Māori Advisory Group to ensure our study is culturally appropriate for Māori.

ETHICS AND DISSEMINATION

Ethics approval for the study was granted by the Central Health and Disability Ethics Committee on 24 August 2021 (reference number: 21/CEN/211). The study protocol adheres to the ethical guidelines outlined in the Declaration of Helsinki.[67] Involvement in this trial will be entirely voluntary. If a recipient agrees to take part, they will be free to withdraw from the study at any time. In addition, the participant will be withdrawn if the research team believes their ongoing involvement in the study is not in their best interest. Donors and recipients will be required to provide written informed consent prior to participation in the study. The ethics committee requires a yearly progress report that must disclose any protocol violations. Communication to the scientific community will be through high-profile international research meetings, as well as relevant national and regional meetings. We aim to publish findings in high-impact peer-reviewed international journals. Further, the research team will communicate the findings to the general public in NZ and overseas through our institute's communications manager. Relevant findings will be shared with the community in a culturally appropriate manner. Participants will be informed of the findings as soon as the results become available. Study findings will also be presented to members of the autistic community via our ongoing relationship with Autism NZ and Altogether Autism.

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