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Gut microbiota and intestinal rehabilitation: a prospective childhood cohort longitudinal study of short bowel syndrome (the MIRACLS study): study protocol

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ABSTRACT

Introduction Short bowel syndrome (SBS) is the predominant cause of paediatric intestinal failure. Although life-saving, parenteral nutrition (PN) is linked to complications and may impact quality of life (QoL). Most children will experience intestinal rehabilitation (IR), but the mechanisms underpinning this remain to be understood. SBS is characterised by abnormal microbiome patterns, which might serve as predictive indicators for IR. We aim to characterise the microbiome profiles of children with SBS during IR, concurrently exploring how parental perspectives of QoL relate to IR.

Methods and analysis This study will enrol a minimum of 20 paediatric patients with SBS (0–18 years). Clinical data and biological samples will be collected over a 2-year study period. We will apply 16S rRNA gene sequencing to analyse the microbiome from faecal and gut tissue samples, with additional shotgun metagenomic sequencing specifically on samples obtained around the time of IR. Gas chromatography with flame ionisation detection will profile faecal short-chain fatty acids. Plasma citrulline and urinary intestinal fatty acid binding proteins will be measured annually. We will explore microbiome–clinical covariate interactions. Furthermore, we plan to assess parental perspectives on QoL during PN and post-IR by inviting parents to complete the Paediatric Quality of Life questionnaire at recruitment and after the completion of IR.

Ethics and dissemination Ethical approval was obtained from the East Midlands—Nottingham 2 Research Ethics Committee (22/EM/0233; 28 November 2022). Recruitment began in February 2023. Outcomes of the study will be published in peer-reviewed scientific journals and presented at scientific meetings. A lay summary of the results will be made available to participants and the public.

Trial registration number ISRCTN90620576.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Short bowel syndrome (SBS) is recognised as the primary cause of paediatric intestinal failure, and while life-saving, parenteral nutrition (PN) is associated with severe complications. Children with SBS demonstrate abnormal gut microbiome profiles compared with healthy children. Intestinal rehabilitation (IR) is a process involving structural and functional changes in the remaining bowel, leading to reduced PN dependence in most children with SBS.

WHAT THIS STUDY ADDS

⇒ The gut microbiome may have a role in intestinal adaptation. This will be the largest cohort study to date of the changes in the gut microbiome in paediatric SBS, with a focus on the potential role of the gut microbiome in predicting successful outcomes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Identifying the microbiota characteristics linked with subsequent IR will inform and justify further study to identify potential therapeutic options for hastening IR. These may include focused enteral nutrition strategies targeted to enhance specific gut bacteria, targeted prebiotic or probiotic use to introduce or enhance specific gut bacteria, and more precise recommendations on types of antibiotics to minimise negative impacts on gut microbiota. Faecal microbiota transplantation with known required beneficial taxa may also be possible. Such strategies have the potential to reduce SBS-associated mortality and PN-associated morbidities and improve quality of life while reducing treatment costs among the growing population of children with SBS in the UK. Additionally, insights into parental perspectives on quality of life and family impact may influence policies surrounding the management of paediatric patients with SBS, aiming to enhance overall care and outcomes.

INTRODUCTION

Short bowel syndrome

Intestinal failure (IF) is defined as the '…critical reduction of the gut mass or its function below the minimum needed to absorb nutrients and fluids required for adequate growth'.[1] IF can be classified functionally into type I (acute, short term and self-limiting), type II (prolonged acute condition, requiring intravenous supplementation over weeks or months) and type III (chronic, metabolically stable, requiring intravenous supplementation over months or years).[2] Short bowel syndrome (SBS) due to IF (SBS-IF) is the most prevalent cause of paediatric IF, with most cases presenting in the neonatal period.[3] SBS is generally defined as IF requiring following extensive bowel resection.[2 3] Underlying surgical conditions vary, the predominant being necrotising enterocolitis (NEC) in about half of affected children.[3]

In the UK, 15 per million children are receiving long-term parenteral nutrition (PN) at home due to SBS.[3] Notably, the prevalence of SBS has surged tenfold over the past 30 years, primarily attributed to advancements in neonatal care, the establishment of specialised regional IF teams and the evolution of PN formulations that have substantially improved long-term survival.[2–4] PN is life-saving but associated with severe potentially fatal complications, notably central line-associated bloodstream infection (CLABSI) and IF-associated liver disease (IFALD), underscoring the imperative to evaluate the longer-term impact on families and the heightened demand for healthcare resources.[4]

Intestinal rehabilitation

Intestinal rehabilitation (IR) is characterised by structural and functional adaptations in the remaining bowel, enhancing absorptive capacity and leading to the discontinuation of PN with the achievement of intestinal autonomy.[5] While the majority of children attain this milestone within the first year of life, 10% continue to require PN beyond 5 years.[6] Beyond the neonatal period, all-cause mortality remains higher than in the general population. The British Artificial Nutrition Survey reports death in around 1 in 60 children with SBS.[3] Liver disease and sepsis are important causes of mortality in this population.[7] Mortality and morbidity may be prevented with earlier IR.[3 7 8]

Predicting the likelihood of achieving IR is challenging, primarily due to the rarity and heterogeneity of the condition. Anatomical considerations, including remaining bowel type, length and underlying disease aetiology, have been associated with successful IR outcomes. Dietary factors influencing the likelihood of IR include the type, timing and mode of nutrient delivery. There is no universally accepted strategy as there are different approaches depending on the centre and/or clinician responsible for the child's care.[7 9] Enterotrophic factors, such as glucagon-like peptide 2 (GLP2), synthesised by enteroendocrine L cells of the distal small intestine and colon in response to enteral nutrients, promote intestinal adaptation. Although GLP2 analogues are licensed for use in paediatric SBS patients, their efficacy is variable and unpredictable, pointing towards the presence of other modifiable factors.[10]

Gut microbiome

The metabolic activity of the gut microbiome is a potential influencer of IR. In contrast to the human genome's approximately 23 000 genes, the gut microbiome encodes over 3 million genes. These micro-organisms maintain a symbiotic relationship with the host, influenced by dietary, environmental and pharmacological factors. Metabolites produced by the gut microbiome are important for host health. For example, short-chain fatty acids (SCFAs) are produced by fermentation of dietary fibre by anaerobic bacteria and contribute to host energy, stimulate motility, promote vascular flow and sodium absorption, prevent growth of opportunistic pathogens, and impact on host–barrier function.[5 11]

Dysbiosis, characterised by disruption to perceived 'normal' taxa diversity, composition and abundance, is linked to disease.[12] Evidence to date suggests significant differences in the microbiome between children with SBS on PN compared with those that have achieved IR, and compared with healthy controls.[5 13–18] These differences involve reduced gut microbial diversity, an increase of bacteria associated with inflammation, for example, Proteobacteria, especially Enterobacteriaceae, and a decrease of bacteria associated with beneficial effects, for example, Bacteroidetes.[5 19–26] Furthermore, SBS patients do not undergo a maturational progression of the gut microbiome as seen in healthy children.[20]

Parenteral nutrition

While PN is a life-saving intervention for children with IF, it imposes a substantial care burden on families, requiring approximately 30 hours per week for direct medical care at home.[27] Children on long-term PN due to IF can suffer from impaired physical, social and emotional health.[28] Overall disease burden is high, marked by the increased number of hospitalisations, procedures requiring general anaesthesia, medical investigations, prescription medications and healthcare visits on PN when compared with those who have weaned from PN.[29 30] Home PN has high-economic costs, with approximately £40 000 prescription expenses per patient per year.[4] A 2006 UK study reported mean costs of £159 000 for children stably maintained on home PN for up to 30 months. With the growing prevalence of children relying on home PN, there is a foreseeable escalating impact on healthcare resources.[31] Efforts that facilitate the successful weaning from PN have the potential to reduce exposure time to complications associated with its use, alleviate the impact on quality of life, healthcare and economic burden associated with prolonged PN dependence.

Aims and hypothesis

We aim to conduct a study in which we will identify and detail the microbiome (population structure and metabolome profile) of children with SBS as they undergo IR.

Specific aims

1. Characterise clinical features of children with SBS who achieve IR and those who do not.
2. Describe differences in gut bacteria (composition and function) as children undergo IR.
3. Compare the gut bacteria (composition and function) between those who have successfully achieved IR and those who remain on PN.
4. Explore parental perspectives on quality of life of children with SBS in relation to IR.

We hypothesise that the gut microbiome differs between children with SBS who do and do not achieve IR and that temporal changes in gut microbiome composition and function are the major predictors of successful IR.

METHODS AND ANALYSES

Study design

This is a nationwide multicentre prospective study of three large UK paediatric IR units, including both intestinal transplant units, accounting for about half the national SBS paediatric patients on PN: The Royal Victoria Infirmary, Newcastle upon Tyne, Great Ormond Street Hospital for Children, London, and Birmingham Women's and Children's Hospital. All potential participants will be identified and recruited by their local IF teams. An estimated 70 children will be identified as eligible across study sites, with target recruitment of at least 20. The overall start date is 1 September 2021 and end date 1 September 2025. The study opened for recruitment in February 2023. See figure 1 for the project overview and figure 2 for project management.

Inclusion criteria

- Age 0–18 years.
- Age >4 weeks post-term corrected gestational age with PN-dependent IF due to SBS or have successfully achieved IR in the year prior to study start.
- Written informed consent.

Exclusion criteria

- Withheld consent.

Recruitment and informed consent

Local clinical teams, serving as both the research team and duly trained National Institute for Health and Care Research (NIHR) Good Clinical Practice healthcare professionals, will identify and approach potential participants. This will occur either during a routine clinic appointment or, for in-patients, on the hospital ward, with patients being invited to consider participation in the study.

Written consent to participate will be taken. A patient information sheet specific to the study will be given to parents with the option of an email copy. Potential participants will have the opportunity to ask questions

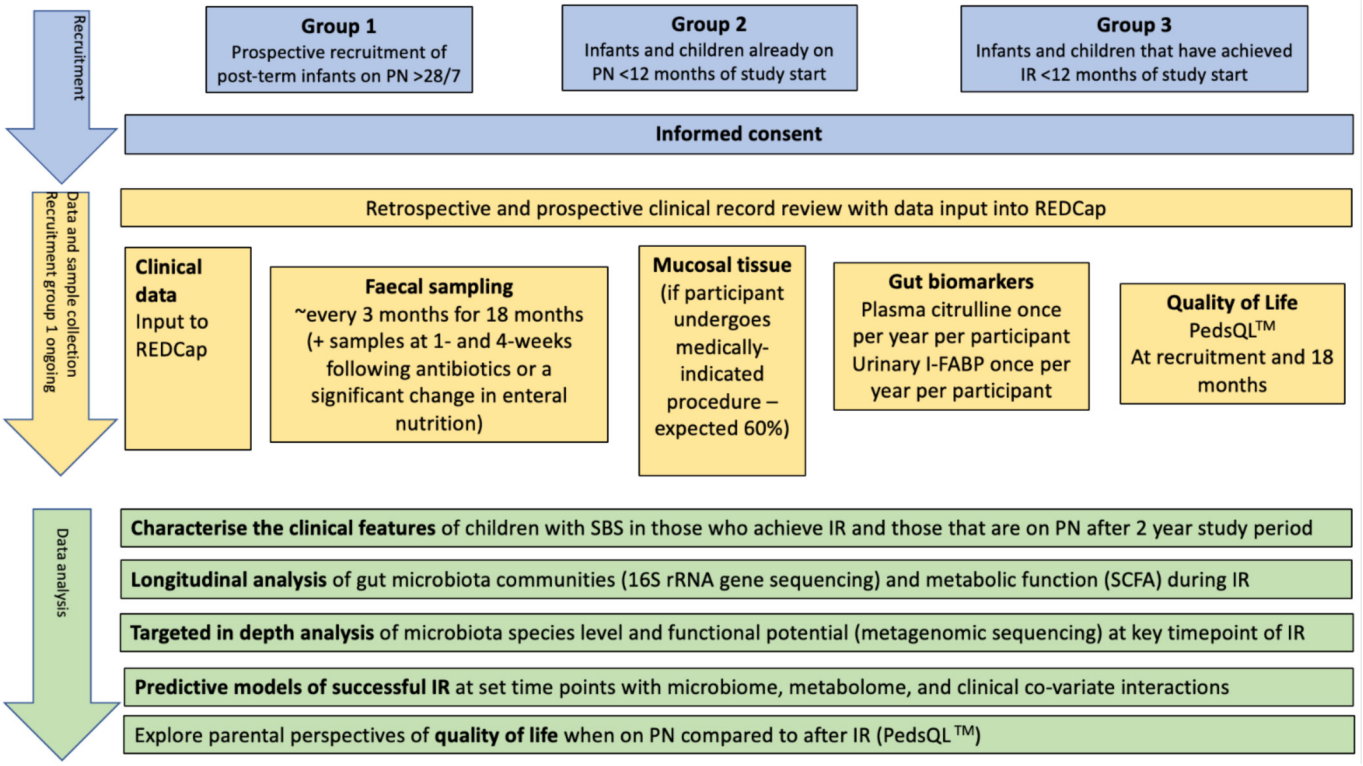

Figure 1 Project overview. I-FABP, intestinal fatty acid binding protein; IR, intestinal rehabilitation; PN, parenteral nutrition; SBS, short bowel syndrome; REDCap, Research Electronic Data Capture ; SCFA, short-chain fatty acid.

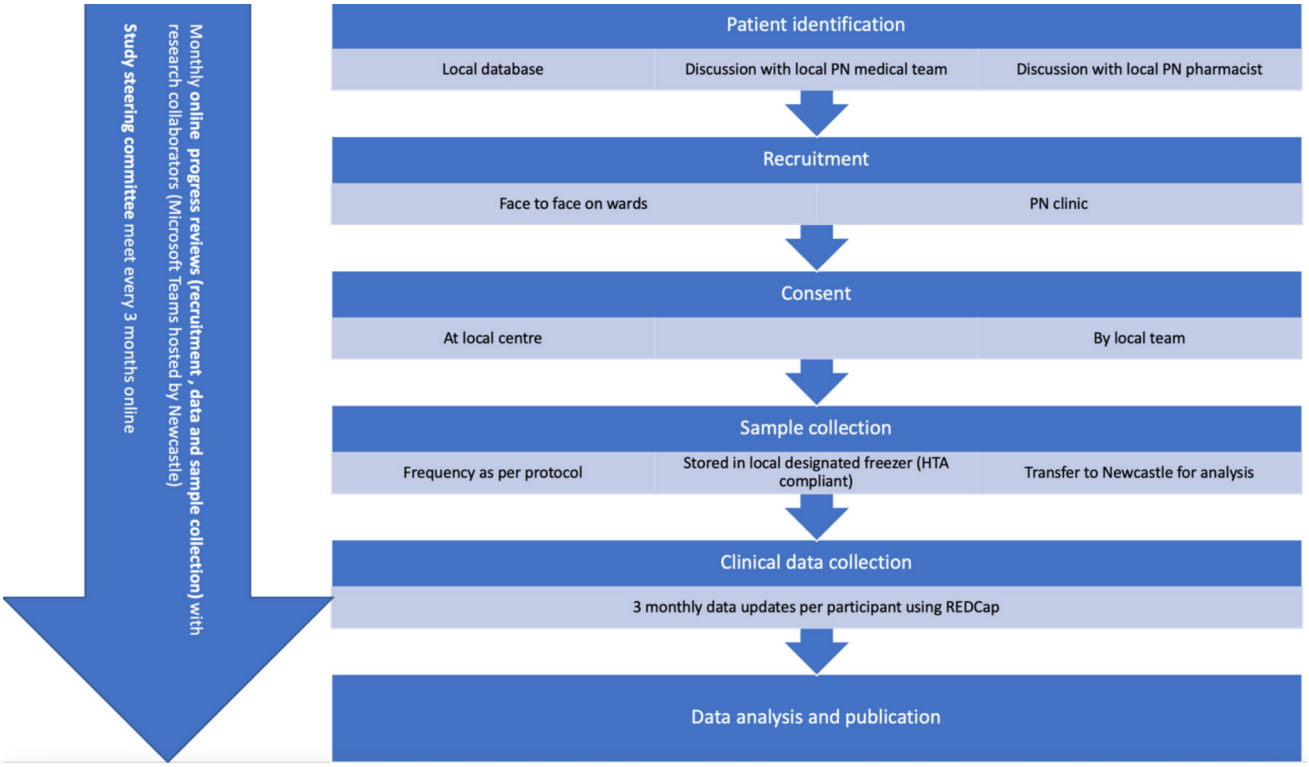

Figure 2 Project management. PN, parenteral nutrition; HTA, Human Tissue Authority; REDCap, Research Electronic Data Capture.

at their clinic appointment and/or at a follow-up telephone appointment. Parents will be invited to provide informed consent either in a clinic or via telephone and postage of completed consent forms. Where deemed appropriate, an age-appropriate patient information sheet will be provided to the child and informed assent or consent, where appropriate, received at the same time. Interpreters will be used in cases where English is not a first language. We are sensitive to the individual choices of patients and families and will not apply any undue pressure.

Outcome measures

Primary outcome measure

- IR (weaning off PN to full enteral feeds for >28 days).

Secondary outcome measures

- Gut bacterial structural and functional profile in relation to IR.
- Proportion of PN as total nutritional intake measured as a percentage (based on Kcal/kg/day PN as a proportion of daily estimated energy intake) using information obtained from patient medical records at routine PN clinical team reviews at 3-monthly intervals.
- Presence of adverse PN-associated clinical outcomes (culture-confirmed CLABSI, IFALD, catheter-related thrombosis), obtained from patient medical records at 3-monthly intervals.
- Quality of life was measured using the Paediatric Quality of Life (PedsQL) questionnaire after recruitment (baseline) and at the end of the study period.

Data and sample collection

Clinical data

Data will be collected from medical records, including anthropometric data, cause of SBS, estimation of initial SB length, gut surgery, antibiotic use, blood parameters (including plasma citrulline, a biomarker for enterocyte mass, using a cut-off of 10 µg/L[32]), DEXA scans, amount of PN required, detailed dietary information and relevant medical information. Data will be pseudoanonymised, collated, stored and managed using the specifically designed secure web-based platform Research Electronic Data Capture, hosted by Newcastle Joint Research Office.

Faecal samples

Faecal samples will be collected at routine PN clinic appointments (approximately every 3 months). Additional faecal samples will be collected following courses of antibiotics or after significant changes in enteral feed type. Samples will be put into sterile universal containers and stored in a −80°C freezer locally then transferred to Newcastle for analysis.[33] For participants who do not bring a frozen sample to the clinic, a pack will be offered containing a sampling tube containing stabilisation agents (OMNIgene•GUT and OMNIgene•MET) to be posted using a prepaid stamped addressed envelope that adheres to Human Tissue Authority (HTA) legislation.[34]

Gut tissue samples

If the participant requires a clinically indicated gastrointestinal endoscopy, and if appropriate preprocedural consents are in place, up to two additional biopsy samples will be requested for analysis of the gut microbiome. If a patient requires a surgical procedure on the gut, a small sample of the resected tissue margin will be requested for analysis.

Urine samples

Intestinal fatty acid binding protein, a marker of gut mucosal injury,[35] will be collected at recruitment and after 1 year.

Quality of life

Parents will be invited to complete the PedsQL questionnaire at recruitment and after IR, to compare parental perspectives on quality of life and the family impact while on PN and after IR.

Duration of sampling

Patients will be studied for up to a 3-year period, depending on the stage of gut health and IR at recruitment. It is, therefore, expected that most participants will be studied for 2 years. This study is completed once all samples have been obtained and analysed. Following this, where appropriate permissions and informed consents are in place, residual samples will be stored in the Great North Neonatal Biobank, hosted by Newcastle University, for future research (HTA licence no. 12534, ethics approval 15/NE/0334, IRAS 161883).

Sample size

There are few studies on which to base sample size calculations. This study is relatively exploratory in nature but aims to include around half the total SBS annual population. To gauge an estimate of sample size, we have used a SD based on a study of children with SBS on PN (n=5) compared with SBS off PN (n=6).[18] Using related published data to generate an SD for sample size estimate calculations is considered an acceptable method employed by researchers.[36] Participants of this study had a range of SBS aetiology and varying gestational ages at birth, typically reflecting this population. Table 1 shows a sensitivity analysis of the power of the study to detect a true difference in the mean Shannon Diversity between two groups (those that have achieved IR compared with those that remain on PN). The effect of different sample sizes and mean differences on the power is demonstrated (α=0.05). The SD (1.318) was calculated from the n=5 children with SBS on PN. The mean difference in Shannon Diversity between groups was 2.59. These data were used in the two-sample t-test for mean differences with equal variance. It is important to consider that these data are based on a SD from a small study, with different outcomes of interest. For pragmatic reasons, we, therefore, plan to invite potential patients that current funding allows and where microbial differences appear meaningful. Currently, we aim to recruit up to 30 participants with the expectation that 20 or more will have adequate longitudinal sampling and variation in achievement of IR to enable the proposed analyses.

Table 1 Sensitivity analysis of the power of the study to detect a true difference in the mean Shannon Diversity between two groups (those that have achieved IR compared with those that remain on PN)

Sample size (per group)	Mean difference in Shannon Diversity	Power
30	2.5	>0.999
30	2.0	>0.999
30	1.5	0.991
30	1.0	0.824
30	0.5	0.304
20	2.5	>0.999
20	2.0	0.997
20	1.5	0.939
20	1.0	0.647
20	0.5	0.215
10	2.5	0.980
10	2.0	0.894
10	1.5	0.673
10	1.0	0.362
10	0.5	0.127
5	2.5	0.748
5	2.0	0.559
5	1.5	0.354
5	1.0	0.185
5	0.5	0.083

IR, intestinal rehabilitation; PN, parenteral nutrition.

Data analysis and interpretation

Gut microbiome

16S rRNA gene sequence data will be processed using established in-house pipelines[37] and metagenome data will be processed using MetaPhlAn4 and HUMAnN4. Statistical analysis will be performed within R using well-established methods within the laboratory group.[38] We will compare alpha-diversity (eg, Shannon diversity) and beta-diversity (eg, 'adonis'). We will compare differences in the relative abundance of taxa while adjusting for potentially confounding variables using microbiome multivariable association with linear models 2. Longitudinal models will also be generated using linear mixed models (LMMs) and generalised LMMs (GLMMs) using the glmmTMB package. All analyses will be adjusted for multiple comparisons using Benjamini-Hochberg false discovery rate correction. We will test for microbiome correlates of IR and microbiome–clinical covariate interactions.

Targeted metabolomics

Frozen faecal samples will be transferred to the University of Glasgow for analysis. Gas chromatography with flame

ionisation detection will profile SCFAs. We will compare differences in the relative and absolute abundance of SCFAs while adjusting for potentially confounding variables with LMMs and GLMMs using the glmmTMB package. All analyses will be adjusted for multiple comparisons using Benjamini-Hochberg false discovery rate correction. Supervised ordination partial least squares-discriminant analysis and significance analysis of microarray with a Delta threshold of 1.0 to identify specific SCFA discriminating successful IR will be determined using used in MetaboAnalyst.[39] GO and enrichment analysis will be performed using the gprofiler2 package V.0.2.1,[40] with default parameters and a customised genetic background.

Integrated analysis

DMM[41] will be used to cluster samples on the basis of microbial community structure and to determine the community types for all samples. Microbiome and metabolomics data will be further compared using generalised procrustes analysis performed using the vegan package V.2.5-7, with p values obtained using the 'protest' function. Specific taxa and SCFAs will be correlated and we will fit predictive models of successful IR at set time points with microbiome, metabolome and clinical measurements using random forests.

Quality of life

Parents will complete the PedsQL Family Impact Module questionnaire at recruitment and after IR, allowing for a comparison of (1) quality of life and family impact during PN and after IR and (2) between participants who have achieved IR by the study end and those remaining PN dependent. The PedsQL Family Impact Module was selected for this study because it has been specifically developed to measure the impact of paediatric chronic health conditions on parent and family functioning, rather than the impact on the individual and is thus less affected by the age of the affected infant.[42]

Patient and public involvement

The parent-led charity for NEC UK has been involved in the development of this project, contributing to protocol development and review of the manuscript. We have consulted with YPAGne during development of the research protocol. Representatives from both YPAGne and NEC UK are members of the study steering committee. We will continue to engage these groups throughout the project, with guidance from their representatives on the research steering committee, providing feedback on the study and supporting meaningful patient and public involvement.

DISSEMINATION

Study organisation and sponsorship is provided by Newcastle upon Tyne Hospitals NHS Foundation Trust, which includes insurance and indemnity cover.

No significant risks are anticipated for study participants. Samples will be obtained during routine clinic appointments (faecal, urine) or medically indicated procedures (gut tissue, blood) with specific consent sought for research. Consent from parents and assent from children, where appropriate, will be in accordance with Good Clinical Practice. Samples will be handled in accordance with the Human Tissue Act.

Families will receive compensation for non-financial losses (inconvenience, discomfort and time) for the provision of the samples for the study). This has been included in the study protocol following consultation with the regional Young Person's Advisory Group during protocol development. There will be no payment associated with risk and payment will be to the parents directly through the provision of a voucher for a widely available retail outlet. It will be discrete and not strongly promoted, in line with Health Research Authority Ethics Guidance for Payments and Incentives in Research.

To disseminate research findings, researchers will submit results to relevant national and international meetings, adhering to the Strengthening the Reporting of Observational Studies in Epidemiology 2007 Statement—Checklist of items for reports of cohort studies (see online supplemental appendix 2).[43] Scientific manuscripts of research results will be submitted to open-access peer-reviewed scientific journals.

NEC UK and YPAGne will assist with preparation and dissemination of lay summaries to appropriate patient groups, charitable organisations and the public. Researchers will seek additional guidance from the Patient, Carer and Public Involvement Manager at Newcastle Joint Research Office (Newcastle upon Tyne Hospitals NHS Foundation Trust) to discuss other opportunities to disseminate findings. Participants may be informed of where presentations and publications can be accessed but they will not have access to individual data or a participant-level dataset.

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Contributors All authors were substantially involved in the conception, design of the study and analysis plan. JSC drafted the article. All authors critically revised the article for important intellectual content and approved the final version to be published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. JEB is responsible for the overall content as guarantor and accepts full responsibility for the finished work and/or the conduct of the study, had access to the data and controlled the decision to publish.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Ethical approval was obtained from the East Midlands—Nottingham 2 Research Ethics Committee (IRAS project ID 306100; REC reference number 22/EM/0233; approved on 28 November 2022). Recruitment began in February 2023.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available.

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REFERENCES

1 Norsa L, Goulet O, Alberti D, *et al*. Nutrition and Intestinal Rehabilitation of Children With Short Bowel Syndrome: A Position Paper of the ESPGHAN Committee on Nutrition. Part 1: From Intestinal Resection to Home Discharge. *J Pediatr Gastroenterol Nutr* 2023;77:281–97.
2 Pironi L, Arends J, Baxter J, *et al*. ESPEN endorsed recommendations. Definition and classification of intestinal failure in adults. *Clin Nutr* 2015;34:171–80.
3 Wiskin AE, Russell R, Barclay AR, *et al*. Prevalence of home parenteral nutrition in children. *Clin Nutr ESPEN* 2021;42:138–41.
4 Hill S, Ksiazyk J, Prell C, *et al*. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Home parenteral nutrition. *Clin Nutr* 2018;37:2401–8.
5 Neelis E, de Koning B, Rings E, *et al*. The Gut Microbiome in Patients with Intestinal Failure: Current Evidence and Implications for Clinical Practice. *JPEN J Parenter Enteral Nutr* 2019;43:194–205.
6 Jo S-YC, McCallum Z, Shalley H, *et al*. Outcomes of Children With Chronic Intestinal Failure: Experience Over 2 Decades at a Tertiary Paediatric Hospital. *J Pediatr Gastroenterol Nutr* 2019;69:e79–87.
7 Pierret ACS, Wilkinson JT, Zilbauer M, *et al*. Clinical outcomes in pediatric intestinal failure: a meta-analysis and meta-regression. *Am J Clin Nutr* 2019;110:430–6.
8 Norsa L, Artru S, Lambe C, *et al*. Long term outcomes of intestinal rehabilitation in children with neonatal very short bowel syndrome: Parenteral nutrition or intestinal transplantation. *Clin Nutr* 2019;38:926–33.
9 Demehri FR, Stephens L, Herrman E, *et al*. Enteral autonomy in pediatric short bowel syndrome: predictive factors one year after diagnosis. *J Pediatr Surg* 2015;50:131–5.
10 Jeppesen PB, Gabe SM, Seidner DL, *et al*. Factors Associated With Response to Teduglutide in Patients With Short-Bowel Syndrome and Intestinal Failure. *Gastroenterology* 2018;154:874–85.
11 Neelis EG, de Koning BAE, Hulst JM, *et al*. Gut microbiota and its diet-related activity in children with intestinal failure receiving long-term parenteral nutrition. *JPEN J Parenter Enteral Nutr* 2022;46:693–708.
12 Durack J, Lynch SV. The gut microbiome: Relationships with disease and opportunities for therapy. *J Exp Med* 2019;216:20–40.
13 Zeichner SL, Mongodin EF, Hittle L, *et al*. The bacterial communities of the small intestine and stool in children with short bowel syndrome. *PLOS ONE* 2019;14:e0215351.
14 Piper HG, Fan D, Coughlin LA, *et al*. Severe Gut Microbiota Dysbiosis Is Associated With Poor Growth in Patients With Short Bowel Syndrome. *J Parenter Enteral Nutr* 2017;41:1202–12.
15 Neelis EG, de Koning BAE, Hulst JM, *et al*. Gut microbiota and its diet-related activity in children with intestinal failure receiving long-term parenteral nutrition. *J Parenter Enteral Nutr* 2022;46:693–708.
16 Korpela K, Mutanen A, Salonen A, *et al*. Intestinal Microbiota Signatures Associated With Histological Liver Steatosis in Pediatric-Onset Intestinal Failure. *J Parenter Enteral Nutr* 2017;41:238–48.
17 Dowhaniuk JK, Szamosi J, Chorlton S, *et al*. Starving the Gut: A Deficit of Butyrate in the Intestinal Ecosystem of Children With Intestinal Failure. *JPEN J Parenter Enteral Nutr* 2020;44:1112–23.
18 Engstrand Lilja H, Wefer H, Nyström N, *et al*. Intestinal dysbiosis in children with short bowel syndrome is associated with impaired outcome. *Microbiome* 2015;3:18.
19 Piper HG. Intestinal microbiota in short bowel syndrome. *Semin Pediatr Surg* 2018;27:223–8.
20 Thänert R, Thänert A, Ou J, *et al*. Antibiotic-driven intestinal dysbiosis in pediatric short bowel syndrome is associated with persistently altered microbiome functions and gut-derived bloodstream infections. *Gut Microbes* 2021;13:1940792.
21 Zhang T, Wang Y, Yan W, *et al*. Microbial alteration of small bowel stoma effluents and colonic feces in infants with short bowel syndrome. *J Pediatr Surg* 2020;55:1366–72.
22 Piper HG, Coughlin LA, Nguyen V, *et al*. A comparison of small bowel and fecal microbiota in children with short bowel syndrome. *J Pediatr Surg* 2020;55:878–82.
23 Phyo LY, Singkhamanan K, Laochareonsuk W, *et al*. Fecal microbiome alterations in pediatric patients with short bowel syndrome receiving a rotating cycle of gastrointestinal prophylactic antibiotics. *Pediatr Surg Int* 2021;37:1371–81.
24 Davidovics ZH, Carter BA, Luna RA, *et al*. The Fecal Microbiome in Pediatric Patients With Short Bowel Syndrome. *JPEN J Parenter Enteral Nutr* 2016;40:1106–13.
25 Wang P, Wang Y, Lu L, *et al*. Alterations in intestinal microbiota relate to intestinal failure-associated liver disease and central line infections. *J Pediatr Surg* 2017;52:1318–26.
26 Engelstad HJ, Barron L, Moen J, *et al*. Remnant Small Bowel Length in Pediatric Short Bowel Syndrome and the Correlation with Intestinal Dysbiosis and Linear Growth. *J Am Coll Surg* 2018;227:439–49.
27 Belza C, Ungar WJ, Avitzur Y, *et al*. Carrying the Burden: Informal Care Requirements by Caregivers of Children with Intestinal Failure Receiving Home Parenteral Nutrition. *J Pediatr* 2022;250:75–82.
28 Hukkinen M, Merras-Salmio L, Pakarinen MP. Health-related quality of life and neurodevelopmental outcomes among children with intestinal failure. *Semin Pediatr Surg* 2018;27:273–9.
29 Han SM, Knell J, Henry O, *et al*. Long-Term Outcomes and Disease Burden of Neonatal Onset Short Bowel Syndrome. *J Pediatr Surg* 2020;55:164–8.
30 Kosar C, Steinberg K, de Silva N, *et al*. Cost of ambulatory care for the pediatric intestinal failure patient: One-year follow-up after primary discharge. *J Pediatr Surg* 2016;51:798–803.
31 Longworth L, Young T, Beath SV, *et al*. An economic evaluation of pediatric small bowel transplantation in the United Kingdom. *Transplantation* 2006;82:508–15.
32 Crenn P, Messing B, Cynober L. Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. *Clin Nutr* 2008;27:328–39.
33 Wang Z, Zolnik CP, Qiu Y, *et al*. Comparison of Fecal Collection Methods for Microbiome and Metabolomics Studies. *Front Cell Infect Microbiol* 2018;8:301.
34 Williams GM, Leary SD, Ajami NJ, *et al*. Gut microbiome analysis by post: Evaluation of the optimal method to collect stool samples from infants within a national cohort study. *PLOS ONE* 2019;14:e0216557.
35 Derikx JPM, Evennett NJ, Degraeuwe PLJ, *et al*. Urine based detection of intestinal mucosal cell damage in neonates with suspected necrotising enterocolitis. *Gut* 2007;56:1473–5.
36 Lenth RV. Some Practical Guidelines for Effective Sample Size Determination. *Am Stat* 2001;55:187–93.
37 Stewart CJ, Ajami NJ, O'Brien JL, *et al*. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature New Biol* 2018;562:583–8.
38 Beck LC, Masi AC, Young GR, *et al*. Strain-specific impacts of probiotics are a significant driver of gut microbiome development in very preterm infants. *Nat Microbiol* 2022;7:1525–35.
39 Pang Z, Chong J, Zhou G, *et al*. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res* 2021;49:W388–96.
40 Kolberg L, Raudvere U, Kuzmin I, *et al*. gprofiler2 -- an R package for gene list functional enrichment analysis and namespace conversion toolset g:Profiler. *F1000Res* 2020;9:ELIXIR-709.
41 Holmes I, Harris K, Quince C. Dirichlet multinomial mixtures: generative models for microbial metagenomics. *PLoS One* 2012;7:e30126.
42 Varni JW, Sherman SA, Burwinkle TM, *et al*. The PedsQL Family Impact Module: preliminary reliability and validity. *Health Qual Life Outcomes* 2004;2:55.
43 Elm E von, Altman DG, Egger M, *et al*. Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007;335:806–8.