Gut microbiota and intestinal rehabilitation: a prospective childhood cohort longitudinal study of short bowel syndrome

The MIRACLS Study

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This protocol has regard for HRA guidance

Research team

Researcher	Role
Dr Jemma Cleminson	Research Fellow, Newcastle Hospitals
Dr Jutta Köglmeier	Consultant in Paediatric Gastroenterology, Great Ormond Street Hospital (Collaborator)
Dr Theo Wong	Consultant in Paediatric Gastroenterology, Birmingham Women's and Children's Hospital (Collaborator)
Dr Christopher Stewart	Wellcome Trust Sir Henry Dale Fellow & Lister Institute Prize Fellow, Newcastle University

Dr Janet Berrington	Consultant Neonatal Paediatrician, Newcastle Hospitals
Dr Julian Thomas	Consultant in Paediatric Gastroenterology, Newcastle Hospitals
Dr David Campbell	Consultant in Paediatric Gastroenterology, Newcastle Hospitals
Prof Nicholas Embleton	Consultant Neonatal Paediatrician, Newcastle Hospitals
Prof Andrew Gennery	Consultant in Paediatric Immunology and Haematopoietic Stem Cell Transplantation

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Signature Page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature:

.....

Date:
...../...../.....

Name (please print):

.....

Position:

.....

Chief Investigator:

Date:
15/08/2022

Signature:

Name: (please print):

Andrew R Gennery

Gut **m**icrobiota and **i**ntestinal **r**ehabilitation: **a** prospective **c**hildhood cohort **l**ongitudinal study of **s**hort bowel syndrome (The MIRACLS Study)

Study Steering Committee

Prof Andrew Gennery	Chief Investigator/supervisor
Prof Nicholas Embleton	Co-investigator/supervisor
Dr Julian Thomas	Co-investigator/supervisor
Dr David Campbell	Co-investigator/supervisor
Dr Janet Berrington	Co-investigator/supervisor
Dr Christopher Stewart	Co-investigator/supervisor
Dr Jemma Cleminson	Doctoral Student/Principal researcher
Carly Martin	Home parenteral nutrition nurse
Marie Spruce	NEC UK Charity parent representative
Dr Neil Davidson	Young Person's Advisory Group North England lead

Roles of study steering committee

- 3 monthly meetings:
  - To review the study protocol
  - To offer feedback to the research team
  - To review study progress

Study proposal summary

We propose a multi-centre prospective pilot study of children with early onset short bowel syndrome. The primary outcome is microbiome and metabolome in relation to intestinal rehabilitation. Families will be approached by clinical team members to participate. We will ask participants to provide non-invasive stool samples at 3 monthly intervals and urine samples once per year, usually coinciding with routine clinical appointments, as well as an additional blood test once per year, when they have routine clinically indicated blood tests. If the participant requires a clinically indicated gastro-intestinal endoscopy, and if appropriate pre-procedural consents are in place, we will request that up to two additional small samples are taken for analysis of the gut microbiota. If a patient requires a surgical procedure on the gut, we will request a small sample of the resected tissue margin for analysis. Patients will be studied up to a 3-year period, depending on the stage of gut health and intestinal rehabilitation at recruitment. We therefore expect most participants to be studied for about 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).

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Trial Title	Gut **m**icrobiota and **i**ntestinal **r**ehabilitation: **a** prospective **c**hildhood cohort **l**ongitudinal study of **s**hort bowel syndrome: The MIRACLS Study (Pilot)
Short Title	The MIRACLS Study
Study Design	Prospective longitudinal cohort multi-centre study
Study centres	• Great North Children's Hospital (GNCH), Newcastle • Birmingham Women's and Children's Hospital (BWCH) • Great Ormond Street Hospital (GOSH), London
Trial Participants	Children with early onset short bowel syndrome
Planned Sample Size	Total n=20 – 30 to achieve adequate sampling
Follow up duration	Up to 3 years
Primary outcome	Achieving intestinal rehabilitation (weaning of PN to full enteral feeds for >28 days)
Secondary outcome measures	• Gut bacterial structural and functional profile in relation to intestinal rehabilitation • Proportion of PN as total nutritional intake • Presence of adverse PN-associated clinical outcomes • Quality of life

Role of study sponsor

Newcastle upon Tyne Hospitals NHS Foundation Trust will provide administrative support and access to IT systems, medical records, and storage for the study.

Protocol contributors

- Chief Investigator (Prof A Gennery)

Study design, methodology, oversight and overall control over data collection, analysis, interpretation and writing and dissemination of manuscripts

- Principal Investigator (Prof A Gennery)

Study design, methodology, oversight and overall control over data collection, analysis, interpretation and writing and dissemination of manuscripts

- Principal Researcher (Dr J Cleminson)

Study design, methodology, generation of protocol, REC approval, data collection, analysis, interpretation and drafting manuscripts and results

- Principal Researcher (Prof N Embleton)

Study design, methodology, oversight and overall control over data collection, analysis, interpretation and writing and dissemination of manuscripts

- Principal Researcher (Dr J Thomas)

Study design, methodology, oversight and overall control over data collection, analysis, interpretation and writing and dissemination of manuscripts

- Principal Researcher (Dr J Berrington)

Study design, methodology, oversight and overall control over data collection, analysis, interpretation and writing and dissemination of manuscripts

- Principal Researcher (Dr C Stewart)

Study design, methodology, oversight and overall control over data collection, analysis, interpretation and writing and dissemination of manuscripts

- Research collaborator (Dr J Köglmeier)

Study design, methodology, contribution to data collection, reviewing of manuscripts

- Research collaborator (Dr T Wong)

Study design, methodology, contribution to data collection, reviewing of manuscripts

- Parent Representative (Marie Spruce, NEC UK Registered Charity 1181026)

Study design, methodology, review of manuscript

- Young Person's Advisory Group North England representative (Dr N Davidson)

Advised on the acceptability of research methodologies, development of important outcome measures to include, how to optimise engagement from families throughout the study, and important factors to include in the patient-information leaflet

Key words

- Paediatric
- Short bowel syndrome
- Parenteral nutrition
- Intestinal rehabilitation

- Microbiota
- Metabolome

Background and rationale

Short bowel syndrome

In short bowel syndrome (SBS) the gut length is too short to sustain health, requiring parenteral nutrition (PN) (1). It is the commonest cause of paediatric intestinal failure (2). Most cases occur neonatally (2). Prevalence has increased 10-fold, as developments in neonatal care and PN improve neonatal survival. Around 75% wean off PN by 3 years but 10% rely on PN beyond age 5 (3). Morbidity relates to duration of PN, as do healthcare costs (4), and child and family's quality of life.

Intestinal rehabilitation

Intestinal rehabilitation (IR) occurs when the remaining intestine adapts to grow and increase function (1). Enteral nutrition is important in IR, but high quality evidence on nutritional interventions is lacking (5). Surgical strategies increase IR but carry significant risk, and intestinal transplant can be lifesaving but requires lifelong immunosuppression (4). Effective non-surgical therapeutic interventions to facilitate IR are restricted to Teduglutide (GLP2 analogue) which is expensive, and its efficacy is variable and unpredictable (6).

Gut microbiota

The human gut microbiota plays a fundamental role in health. Children with SBS show reduced gut microbial diversity compared to healthy controls, with an increase of bacteria associated with inflammation and a decrease associated with beneficial effects (1, 7), which persists into early childhood. Furthermore, SBS patients do not undergo maturational progression of gut microbiota as seen in healthy children (8). Small cross-sectional studies show reduced gut bacterial diversity in children with SBS on PN compared to those that have achieved IR (1). The gut microbiome has huge metabolic functional potential. Short chain fatty acids (SCFAs) are important metabolites produced by fermentation of fibre by anaerobic bacteria. They contribute to host energy, stimulate motility, promote vascular flow and sodium absorption, prevent growth of opportunistic pathogens, and impact on host barrier function (1, 9). One small study that included one child with SBS who weaned from PN during the study period demonstrated that gut microbiota became more diverse, with increased proportions of SCFA producing Bacteroidetes and Bifidobacteria after IR compared to prior (9). Children receiving enteral nutrition have greater gut microbial diversity (10). Enteral nutrition may therefore have an important relationship with gut microbiota during IR. Shotgun metagenomic sequencing techniques show differences in the genetic potential between patients receiving PN and healthy controls, including depleted carbohydrate metabolism pathways and an increase in antibiotic resistance genes (1, 8).

There is significant need for a comprehensive longitudinal study combining clinical data and biological samples to provide an understanding of the temporal changes in gut microbiota associated with timing and success of IR.

Project Development

Our Newcastle study of 31 children with early onset SBS treated over 11 years showed that antibiotic use was associated with reduced likelihood of achieving IR, suggesting that intestinal microbiota play a key role (11). In a REC approved feasibility study of our proposed methodology, we performed 16S rRNA sequencing of faecal samples from five patients (4 dependent on PN, median age 51.5 months (range 36.5-61.5)) and one who had achieved IR (age 120 months), showing a higher relative abundance of *Lactobacillus* after IR compared to those on PN, in keeping with published data and demonstrates study feasibility (see Figures 1 and 2) (1). *Lactobacillus* impacts host barrier integrity and function and is potentially modifiable through enteral feed type and probiotic supplementation.

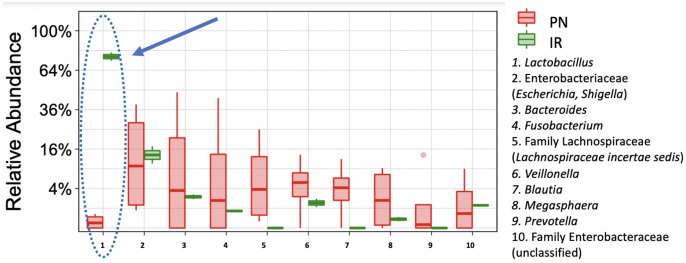

Figure 1 *Box plot of gut bacterial taxa abundance demonstrating higher abundance of Lactobacillus in IR compared to PN*

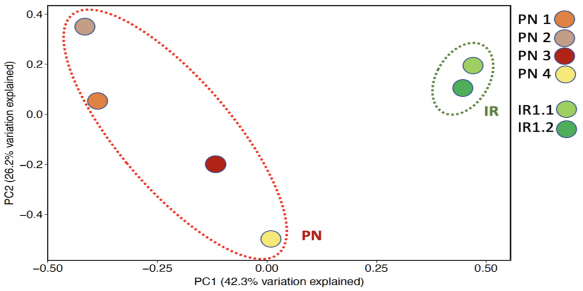

Figure 2 *Bray-Curtis PCoA of microbiome profiles demonstrating dissimilarity of gut bacterial communities between IR and PN*

We have established a study steering committee, including YPAGne and parental charity NEC UK, to inform outcome measures, acceptability of study protocols and advice on patient information sheets. We have developed a secure online data collection tool to share participant data adhering to relevant governance and ethical requirements (REDCap).

Improving understanding of longitudinal changes in the gut the microbiome during IR offers significant potential in understanding and facilitating IR. Current evidence is from very small cross-sectional cohort studies. Combining clinical data, biological samples, and sophisticated analytic techniques will provide an understanding of the temporal changes in gut microbiota associated with timing and success of IR. Identifying the microbiota characteristics linked with subsequent IR presents potential therapeutic options for hastening IR, such as specific enteral nutrition strategies including selected pre- and pro-biotic use to benefit gut microbiota, and more judicious use of antibiotics to reduce negative impact on the gut microbiota, thereby reducing PN-associated morbidities, improving quality of life, and improving survival, whilst reducing treatment costs, amongst the growing population of children with SBS in the UK.

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Hypothesis

The gut microbiota differs between children with SBS who do and do not achieve IR. Temporal changes in gut microbiota composition and function are the major predictors of successful IR amongst children with early onset SBS.

Research questions

Principal research question

Are differences in the gut microbiota a key factor in determining successful intestinal rehabilitation in children with early onset short bowel syndrome?

Secondary research questions

1. What are the clinical characteristics, including patterns of markers of gut function, of patients with SBS that achieve IR?
2. Does the gut microbiota composition change during IR?
3. Does the gut microbiota function change during IR?
4. How do courses of systemic antibiotics impact on the gut microbiota and natural history?
5. How do significant changes in enteral feeds impact on the gut microbiota?
6. How does the gut microbiota at mucosal level compare to that in faeces in children with early onset SBS?
7. Could we identify possible therapeutic interventions that may reduce the time to IR and be amenable to a prospective RCT?
8. Is there an association between quality of life and achieving IR?

Aim

To conduct a study in which we will identify and detail the microbiota (population structure and metabolome profile) of children with early onset short bowel syndrome as they undergo intestinal rehabilitation.

Our specific aims are to:

1. Characterise clinical features of children with early onset SBS who achieve IR and those that 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 that 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

Proposed study plan

We propose a cross-disciplinary collaborative multicentre prospective study of three large UK paediatric IR units, including both intestinal transplant units, accounting for ~ half the national SBS

paediatric patients on PN. An estimated 70 children will be identified as eligible across study sites, with estimated recruitment of at least 20.

Figure 3 and *Figure 4* provide the project overview and study co-ordination plan. This will draw on the expertise of all applicants. Dr Cleminson is a Doctoral student at Newcastle University and will undertake this project with supervision from the PI and co-applicants. Meetings with supervisors at Newcastle will be held monthly and data sharing with the research team (including collaborators across study sites) will be held periodically to discuss project recruitment, sampling provision, data collection and outcomes. Quarterly, or as required, study steering committee meetings will be held to discuss project outcomes.

Inclusion criteria

- Group 1: Prospective recruitment of infants born within the study period from 4 weeks post-term with PN-dependent intestinal failure due to short bowel syndrome
- Group 2: Any child already on PN and meets criteria for group 1 in the year before the study commences
- Group 3: Children who had SBS but have successfully achieved IR in the year before study commences
- Group 4: Preterm infants <28/40 at birth on PN at term CGA (retrospective and prospective, these infants will have permissions for study under the SERVIS study)

Exclusion criteria

- Informed consent not provided

Primary outcome measure

- Achieving intestinal rehabilitation (weaning of PN to full enteral feeds for >28 days)

Secondary outcome measures

- Gut bacterial structural and functional profile in relation to intestinal rehabilitation
- Proportion of PN as total nutritional intake
- Presence of adverse PN-associated clinical outcomes
- Quality of life

Data collection (all participants)

Study data will be collected during routine clinic visits and recorded in a pseudo-anonymised database (REDCap) and include but is not limited to:

- Demographic details, including:
  - Date of birth
  - Gestation at birth
  - Sex
  - Mode of delivery

  - Use of perinatal antibiotics
- Clinical details, including:
  - Cause of SBS
  - Age of onset
  - Surgeries at SBS onset (including estimation of SB length)
  - Bowel lengthening procedures
  - Antibiotic use (Indications, dates, type)
  - Central line infections (Site, organism, sensitivities, treatment)
  - Dates of central line changes/insertion and indication
  - Number of hospital admissions and reasons for them
  - Co-morbidities
- Nutritional input details, including:
  - PN requirement (volume, composition, frequency of administration, duration)
  - Enteral dietary intake from birth (including, type and method)
- Anthropometric data
  - Weight
  - Height
  - BMI
  - Head circumference
  - Mid-upper arm circumference
  - Skin fold thickness
- Haematological and biochemical parameters, including:
  - Full blood count
  - Urea and electrolytes
  - Liver function tests
  - Bone profile
  - CRP, ESR
  - Micronutrients
  - Haematinics
  - Coagulation screen
  - Vitamin status
  - Plasma citrulline
  - Faecal calprotectin
  - Immunological parameters
- Radiology

- DEXA scans, routinely undertaken at 1-3 yearly intervals on these patients, will be included where available

Patients usually attend routine clinics about 3 monthly, where these data are routinely collected.

Data will be recorded in a pseudonymised database (Research Electronic Data Capture (REDCap)) hosted by the Newcastle Joint Research Office (NJRO).

All patient related study data will be collected during routine clinic visits.

Faecal samples

Faecal samples: Samples will be analysed for microbial composition and SCFAs. Non-invasive faecal samples will be collected from each child at routine clinic appointments (approximately at 3 monthly intervals) or during a patient's in-patient admission and linked to detailed clinical data as described above. We recognise that faecal sample provision is opportunistic. Therefore, we will request that parents obtain the sample within 72 hours prior to the clinic visit and store it in plastic bags (provided by the study) in a home freezer, if available.

To support families with sample provision, timely reminders will be sent by text message within 3 days of the clinic appointment from a research specific mobile telephone at each site. This will be monitored by the local research nurse and researchers. This phone will also provide families with a contact for if they have any queries regarding the sample collection and provision. Posters in the PN clinic room will also act as reminders.

Samples will be labelled with a pseudoanonymised study number, sample number and sample date (no patient-identifiable information) prior to storage in a -80^{o}C University freezer. If required, the samples will be transferred to the Blood Sciences Department for temporary storage until collection by a researcher and then transferred the University freezer as soon as feasible. For samples obtained in Birmingham and London, they will be stored in a -80^{o}C freezer until transfer to Newcastle for analysis.

For participants who do not bring a frozen sample to clinic, a pack will be offered containing a sampling tube containing DNA stabilisation agent (OMNIgene®•GUT) to be posted using a prepaid stamped addressed envelope that adheres to HTA legislation (12).

Gut tissue samples

For children requiring endoscopy or gastrointestinal surgery for clinical reasons (expected in about 60% of participants), we will seek consent for up to 2 additional biopsy samples or use of leftover tissue. The DNA yield from 1 biopsy can be small, so taking up to 2 biopsies (at the discretion of the surgeon) helps ensure sufficient biological material without additional significant risk to the patient.

Next generation sequencing

Samples will be homogenised, DNA will be extracted and 16S rRNA gene sequencing performed. Patients who achieve IR will undergo shotgun metagenomic sequencing of their final stool sample

prior to IR and results compared to patients that have remained on PN for a similar duration (expected for about one third). Where funding resources allow, we will perform shotgun metagenomic sequencing on faecal samples at additional key timepoints (prior to IR and post IR) to explore longitudinal changes in metabolic functional potential. We will perform blank extractions to account for potential contamination. Performing 16S rRNA gene sequencing is a cost-effective approach to enable longitudinal analysis of the microbiome, as well as performing an amplification step, which will be important for identifying low abundance bacteria. Performing more expensive metagenomics on a targeted cross-sectional subset of stool samples will allow identification to bacterial species level and provide important information on microbial functional potential prior to IR.

Urine samples

Urine for Intestinal fatty acid binding protein (I-FABP, a marker of gut mucosal injury, inflammation, and maturation (13), will be collected at recruitment and approximately yearly thereafter.

Blood samples

An additional sample for plasma citrulline will be taken at the time of routine blood sampling once per participant per year. Serum citrulline is a biomarker for small intestinal enterocyte mass. Sampling will be standardised, using a cut off of 10microgram/l (14) (15, 16). Children with SBS are not fasted overnight and may receive enteral feeds in the day so we will endeavour to make the timing of samples as standardised as possible. The PN clinics run at the same time each week, so samples will be obtained for plasma citrulline when children undergo routine monitoring blood tests during these clinics (15, 16). For participants at Birmingham, plasma citrulline is monitored annually as part of routine care.

Quality of life

Parents will be invited to complete the PedsQL^{TM} questionnaire with additional free text comments at recruitment and at the end of the data collection period, to compare parental perspectives on quality of life and the family impact whilst on PN and, if achieved, after IR.

Sample Size

There are few studies on which to base sample size calculations. This study is therefore still relatively exploratory in nature but will include around half the total SBS annual population. However, to gauge an estimate of sample size, we have used microbial measures and a standard deviation based on a small study of children with SBS on PN (n=5) compared to SBS off PN (n=6) (17). Using related published data to generate a standard deviation for sample size estimate calculations is considered an acceptable method employed by researchers (2). *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 to those that remain on PN). The effect of different sample sizes and mean differences on the power are demonstrated (α =

0.05). The standard deviation (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 2-sample t-test for mean differences with equal variance. It is important to consider that these data are based on a standard deviation 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 the proposed analyses.

Sample size (per group)	**Mean difference in Shannon Diversity**	**Power**
30	2.5	>.999
30	2.0	>.999
30	1.5	0.991
30	1.0	0.824
30	0.5	0.304
20	2.5	>.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

Table 1 Sensitivity analysis

Data analysis and interpretation

Sample selection: depending on patient sampling longitudinally and measures of IR we will select the most suitable well sampled infants and samples for the described analyses and comparisons that budgetary constraints allow. We are currently funded for a minimum of 20 participants.

Gut microbiota: 16S rRNA gene sequences will be processed using established in-house pipelines and metagenome data will be processed using MetaPhlAn2 and HUMAnN. Statistical Analysis will be performed within R using well-established methods within the laboratory of Dr Stewart. We will compare alpha-diversity (e.g., Shannon diversity) and beta-diversity (e.g., adonis). We will compare differences in the relative abundance of taxa while adjusting for potentially confounding variables using MaAsLin2. 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 discover rate correction. We will test for microbiome correlates of intestinal rehabilitation and microbiome-clinical co-variate interactions**.** We will fit predictive models of successful IR at set time points with microbiome, metabolome and clinical measurements using random forests. Dr Stewart's lab has a track-record in supporting researchers to analyse such datasets and will ensure the data are used to their full potential.

Targeted metabolomics: frozen faecal samples will be transferred to Professor Gerasimidis' laboratory at the University of Glasgow for analysis. Gas chromatography with flame ionization detection (GC-FID) will profile SCFAs.

Recruitment

Patients will be identified and approached by the local clinical teams at study sites (who are also the research team) during a routine clinic appointment or, for in-patients, on the hospital ward, to be invited to consider participation in the study.

Consent

Written consent to participate will be taken. The project plan will be explained, as well as what data will be recorded and how data will be stored. A patient information sheet specific to the study will be given to parents to keep for reference, and an email copy will be sent to parents if they wish. The latter was suggested for inclusion in the protocol by NEC UK Charity Chair Trustee Marie Spruce. Potential participants will have the option to ask questions at the clinic appointment and at a follow up telephone appointment. Parents will be invited to provide informed consent either in 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 received at the same time. Interpreters will be used in cases where English is not a first language, or if there is concern from a researcher that understanding is not sufficient to permit informed consent.

We are sensitive to the individual choices of patients and families and will not apply any undue pressure.

Ethical considerations

This project involves the non-invasive collection of faecal, urine, blood, and surgical and endoscopic gut tissue biopsy samples from children under the care of clinical teams based in Newcastle. Collection protocols are an established part of the Newcastle Neonatal team's current studies, including SERVIS (REC 10/H0908/39) and the Great North Neonatal Biobank (REC 15/NE/0334). The Great North Neonatal Biobank was established and is managed by Dr Berrington, with extensive experience using human samples for research. Non-invasive sampling is highly acceptable to parents, and systems exist to allow postage of samples obtained at home. Gut mucosal samples and blood samples will be obtained during medically indicated procedures 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.

Before the start of the study, a favourable opinion will be sought from a Research Ethics Committee for the study protocol, and relevant documents through the Integrated Research Application System (IRAS). In accordance with REC guidance, the Chief Investigator will notify the REC at the end of the study and provide a final report following the end of the study.

Families will receive compensation for non-financial losses (inconvenience, discomfort, and time) for the provision of the samples for the study (£20 per family in the form of retail vouchers). 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.

Amendments

If there are any amendments to the protocol, the principal researcher will action and share these with the Chief Investigator. The amended protocol, with history of amendments documented, will then be submitted to relevant bodies (REC and local R&D departments).

Peer review

We will seek support with obtaining peer review from Newcastle Joint Research Office (Newcastle upon Tyne Hospitals NHS Foundation Trust).

Patient and public involvement

The parent-led charity for necrotising enterocolitis 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.

To ensure that research outcomes are disseminated to relevant patient groups and the public, a lay summary of key research findings will be prepared in conjunction with YPAGne and NEC UK then distributed to appropriate patient and charitable organisations, such as 'Short Bowel Survivor and Friends'. I will attend public engagement activities hosted by these organisations to present key research findings.

Twice yearly, I will meet with the Patient, Carer and Public Involvement Manager at Newcastle Joint Research Office (Newcastle upon Tyne Hospitals NHS Foundation Trust), to discuss upcoming opportunities to disseminate findings with and integrate PPI. Opportunities to disseminate my research in Birmingham and London include the 'Pint of Science' event (also in Newcastle), both of which are geared to engage young people in particular with ongoing scientific research.

Data protection and patient confidentiality

The research group proposed in this fellowship have expertise in the collection, processing, data management and storage with such techniques and samples, and adhere to current relevant codes of practice, including the Human Tissue Act 2004, Data Protection Act 1998, and UK General Data Protection Regulations, with the utmost respect and responsibility. We will ensure that all relevant research ethics committee approvals and regulatory requirements are met and will follow the relevant Codes of Practice issued by the Human Tissue Authority. We will adhere to Caldicott guidelines regarding the collection, transfer, and storage of participant information.

Informed consent will be obtained according to GMC Good Medical Practice guidance. The proposed sampling approaches are well-accepted to families, as samples that are being requested are either non-invasive or taken during a medically indicated procedure. Hence, consent rates are high, and parents are keen to support research that may ultimately result in improved understanding of their child's condition and may result in further studies that translate to improved patient outcomes (18).

Newcastle University's Research Data Service have assisted with development of a Data Management Plan, in accordance with University Policies and relevant national legislation.

Participant data obtained on hospital premises for storage and project purposes will be link-anonymised by research staff prior to transfer. Personal identifiers, both direct and indirect, will be removed. Participant data and samples will be link-anonymised and data stored linked to a unique study number. Details of research participants and their corresponding study number will

be stored in a protected excel sheet accessible only to project researchers. The core study team will have access to the data during the study; this comprises of the principal researcher, chief investigator, and any supplemental clinicians. Only the Chief Investigator and principal researchers will have access to the full dataset.

Hard copy data will be stored in locked offices that are only accessible to research staff for only as long as necessary, prior to digitalisation and secure password-protected access. All data that may be of future use will be stored digitally. Data will be uploaded to REDCap on a regular basis. Access to confidential electronic data will be password-protected accessible to project staff only.

Electronic data will be stored on a secure REDCap database, hosted on a Health and Social Care Network server. Password-protected access will be given to members of the study team; other sites will have internet access allowing input of data only, with no 'read' capability. Sign on credentials will be required to access the server.

Any published material will not include any identifiable data.

Link-anonymised research data that has long-term value will be held for up to 5 years, although if deemed appropriate then it can be stored indefinitely. Where possible all data of long-term value will be digitalised and archived into a repository to ensure access. Personal identifiers, both direct and indirect, will be removed.

Indemnity

Newcastle University's indemnity scheme will apply to NHS sites in this study.

Dissemination Policy

Following data collection, analysis and interpretation, results will be presented to REC and collaborating sites. To disseminate research findings to relevant academic parties and the interested wider audience, researchers will submit results in oral and poster format to relevant national and international meetings. To make research findings accessible worldwide, scientific manuscripts of research results will be submitted to open access peer-reviewed scientific journals that have a broad readership. Authorship will be shared between the researchers and any participating centres. As a member of RCPCH, BSPGHAN and BAPEN professional bodies, which include allied health professionals in their membership, I will request publication of research summaries on their respective websites, to reach a wider clinical audience. 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.

Justification of resources

To ensure the project objectives are met, costs are estimated at a total of £19,160 (based on 20 participants):

- Sample collection, storage, and transfer are done by researchers during routine clinical care so costs will be minimal (~£200 for provision of sample containers)
- General laboratory consumables will cost ~£1000, based on similar recent studies within the research group
- REDCap maintenance for 2 years will cost £5000.
- A study specific mobile phone and mobile data will cost £440
- An estimated 130 faecal samples will undergo microbial DNA extraction and 16S rRNA Sequencing at a cost of ~£3200 (depending on sample provision and number of additional samples required as per protocol)
- For faecal samples that are requested but not brought to clinic, and are of particular importance, OMNIgene®•GUT kits will be provided. We estimate up to £1000 will be required to ensure sample provision.
- Faecal samples from a subset of patients that achieve IR in the study period, and an equal number of (best matched) participants that do not, will undergo Shotgun metagenomic sequencing (estimated n=6 IR and n=6 no IR) at a cost of £1800
- 16S rRNA Sequencing of gut tissue samples (n=12 participants, n=24 samples) will cost £600
- Targeted metabolomics (SCFA analysis) of 100 samples at a cost of ~£2500
- We will aim to analyse, where costs and samples allow, up to 16 – 25 infants for urinary FABP and plasma citrulline (cost ~ (£2000)
- Use of the PedsQL™ questionnaire for the duration of the study will cost ~£800
- Recompense to families for non-financial losses (£20/family as retail vouchers) will cost ~£400
- Courier of samples, with regard to HTA guidance, from London and Birmingham, and from Newcastle to Glasgow, will cost ~£220

Gut **m**icrobiota and **i**ntestinal **r**ehabilitation: **a** prospective **c**hildhood cohort **l**ongitudinal study of **s**hort bowel syndrome (The MIRACLS Study)

Appendix

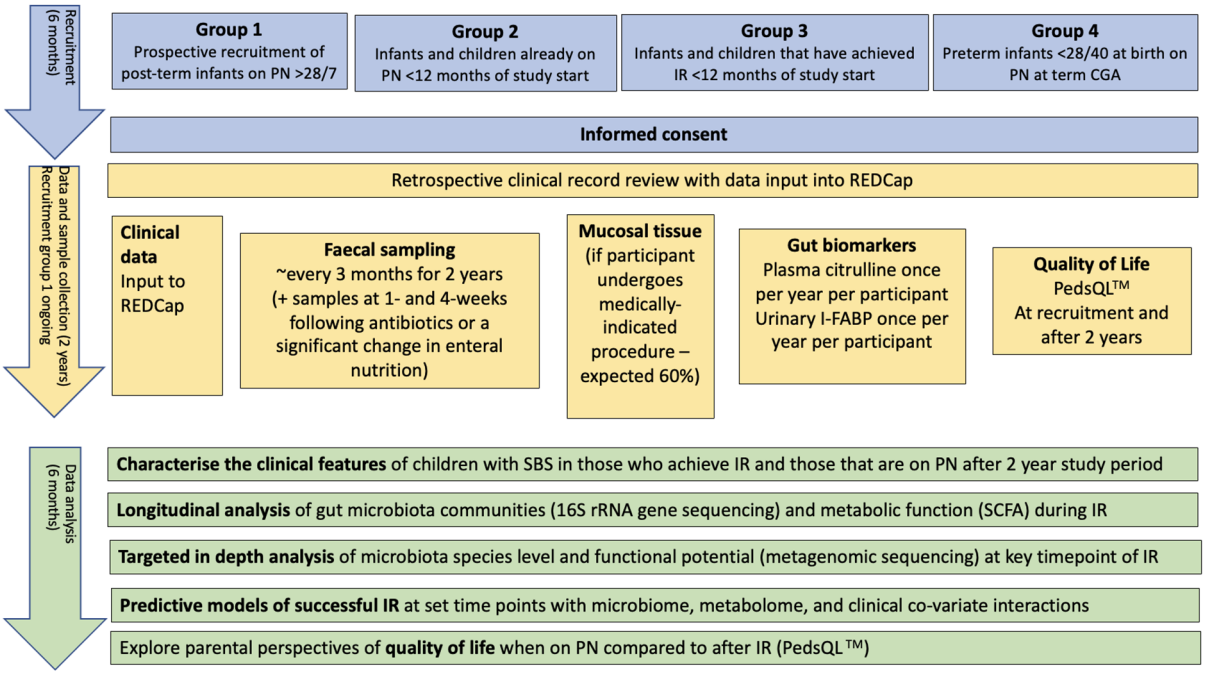

Figure 3 *Project overview*

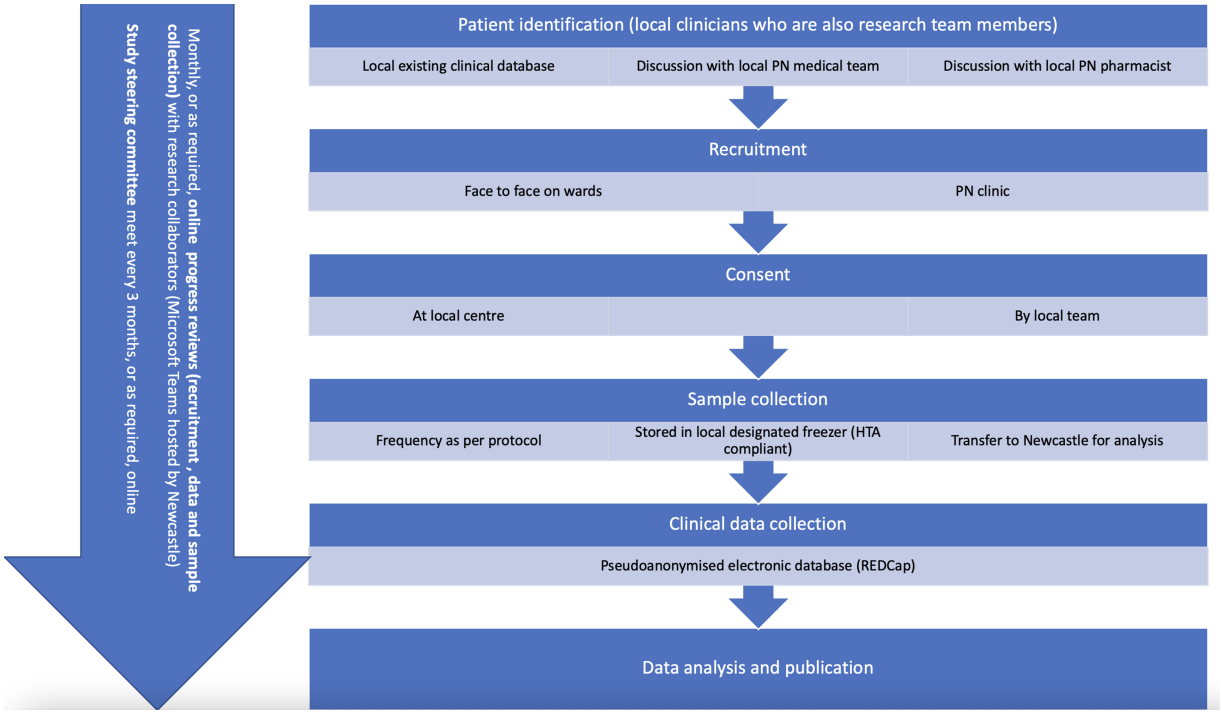

Figure 4 *Project management*

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