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Longitudinal profiling of the gut microbiome in patients with psoriatic arthritis and ankylosing spondylitis: a multicentre, prospective, observational study

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Abstract

Background: Psoriasis is a chronic inflammatory disease of the skin affecting 2–3% of UK population. 30% of people affected by psoriasis will develop a distinct form of arthritis within 10 years of the skin condition onset. Although the pathogenesis of psoriatic arthritis is still unknown, there is a genetic predisposition triggered by environmental factors. Limited but convincing evidence link the gut microbiome to psoriatic arthritis. The Microbiome in Psoriatic ARThritis (Mi-PART) study propose is to characterise the microbiome-metabolic interface in patients affected by psoriatic arthritis to deepen our understanding of the pathogenesis of the disease.

Methods: This is a multicentre, prospective, observational study. Psoriatic arthritis ($n = 65$) and ankylosing spondylitis ($n = 30$) patients will be recruited in addition to a control group of healthy volunteers ($n = 30$). Patients eligibility will be evaluated against the Criteria for Psoriatic Arthritis (CASPAR), the Bath Ankylosing Spondylitis Activity Index (BASDAI) and the healthy volunteers who fulfil study inclusion and exclusion criteria.
Information regarding their medical and medication history, demographics, diet and lifestyle will be collected. All the participants in the study will be asked to complete a 7-day food diary, to provide stool samples and to complete quality of life questionnaires. Routine clinical laboratory tests will be performed on blood and urine samples. Patients and healthy volunteers with gastrointestinal symptoms, previous history of cancer, gastrointestinal surgery in the previous 6 months or alcohol abuse will be excluded from the study.

(Continued on next page)

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(Continued from previous page)
Discussion: The aim of this trial is to characterise the microbiome of psoriatic arthritis patients and to compare it with microbiome of healthy volunteers and of patient with ankylosing spondylitis in order to define if different rheumatologic conditions are associated with characteristic microbiome profiles. Investigating the role of the microbiome in the development of psoriatic arthritis could deepen our understanding of the pathogenesis of the disease and potentially open the way to new therapies.

Keywords: Psoriatic arthritis, Microbiome, Ankylosing spondylitis, Metabolomics, Gut-join axis

Background

Psoriasis is a chronic inflammatory skin disorder that affects 2–3% of UK population [1]. It is characterised by a recurring erythematous papular rash without scars [2]. Psoriasis is associated with several different co-morbidities like a characteristic form of inflammatory arthritis [3]. Clinically, psoriatic arthritis (PsA) is mostly identified by the coexistence of psoriasis and arthritis, however PsA can present different patterns: distal and asymmetric oligoarthritis, symmetric polyarthritis, arthritis mutilans and spondyloarthritis [4]. PsA can induce joints damage quickly with 27% of patients showing irreversible damage in the first year from onset [5].

The pathogenesis of the disease is poorly understood however there is evidence of a genetic component in PsA, it has been demonstrated by studies showing an increased recurrence in first degree relatives [6]. Genes that have been demonstrated associated with an increased risk of developing psoriatic arthritis include HLA-B27, IL 13 and PTPN22, with the strongest association demonstrated for HLA B27 [7]. HLA-B27 is a well-established major risk factor for the development of ankylosing spondylitis (AS), but also 15–20% of PsA patients express HLA-B27 with a stronger association with the axial distribution of the disease [8, 9]. Additional risk factors for psoriatic arthritis include obesity [10], smoking [11], the presence of nail psoriasis and possibly the severity and distribution of psoriasis, some of these factors are linked to the host's microbiome [12, 13].

In conclusion, several studies have demonstrated the presence of a network at the microbiota-metabolite-immunology level in the human setting of PsA, a chronic inflammatory disease [14, 15].

Rationale

A growing number of evidence links alteration of the gut microbiome to the development of autoimmunity [16–18]. Studies conducted on animal models of ankylosing spondylitis using HLA-B27 rats have demonstrated the role of microbiome in inducing the disease [19, 20]. Scher et al. have recently identified a different gut microbiota in PsA patients and a concomitant reduction in short chain fatty acids (SCFA) when compared with healthy controls [21]. All these evidences suggest a link between an altered gut microbiome and PsA [12, 22]. Furthermore, there are several recognisable clinical patterns of PsA that overlap with AS and some cases may be even indistinguishable [4]. The study of the gut microbiome of PsA and AS patients brings an opportunity to compare if there is further differences that allow a more precise diagnosis of both diseases.

Thus, PsA is ideally suited to a phenomic approach of studying the mechanisms by which microorganisms and their metabolites affect immunity – investigating the gut and skin microbiome in the setting of inflammatory arthritis. This project should result in potential biomarkers of disease and putative signalling molecules for pharmacological analysis in the future. Additionally, by undertaking a metabolomic approach to the microbiome, novel therapeutic targets could be identified, and treatment approaches stratified according to the individual patients' genetic and microbiomic drivers of inflammation.

Study Aims

- To stratify PsA and AS patients according to the disease activity and subtypes based on microbial networks.
- To deepen our understanding of psoriatic arthritis and to identify a panel of biomarkers that would allow stratification of patients according to the disease severity and therapy.
- Phenomic characterisation to determine how the microbiome interact with the host and potentially drive disease.
- To test the genetic predisposition to the development of the disease in association with environmental trigger factors.
- Use phenomic data to develop dietary or pharmaceutical interventions.

Methods and analysis

Study design

This is a multicentre, prospective and observational study to collect data and samples from consented males and females of any ethnicity origin aged over 18 years, diagnosed with psoriatic arthritis or ankylosing

Table 1 The study is being recruited into four cohorts. The different number of visits is separated by 12 weeks

Group	Health Status	Number of subjects	Number of visits per subject
Group A	Psoriatic Arthritis	15	Screening, visit 1,2 and 3
Group B	Psoriatic Arthritis	50	Screening and visit 1
Group C	Ankylosing Spondylitis	30	Screening and visit 1
Group D	Healthy volunteers	30	Screening and visit 1

spondylitis or healthy volunteers from two geographical locations.

- Royal National Hospital For Rheumatic Diseases (RNHRD), Bath, UK.
- Hammersmith Hospital, London, UK.

We will recruit a total of 125 subjects (95 PsA, 30 AS) and 30 healthy volunteers (Table 1). This study adheres to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition) [23]. It is also conducted in compliance with the Data Protection Act and the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) guidelines for clinical studies (Table 2).

Participants and recruitment

The patients will be recruited from rheumatology clinics under consultant's advice. Self-referred patients will be involved through advertisements on noticeboards or websites/newspapers. All the questionnaires, indexes and scores mention in this section are detailed in the Additional file 1.

Any male or female patients older than 18 that fulfil the CASPAR or the BASDAI criteria will be invited for screening. They will receive the full patient information sheet (PIS) and informed consent forms (ICF). Potential participants will be allowed to consider full implications of taking part in the study for a minimum of 24 h before screening. They will also have the opportunity to ask questions before signing the consent.

Patients will not be able to take part to the study if they:

- Are not able to give informed consent
- Are pregnant or breastfeeding
- Have a history of alcohol, drugs or chemical abuse in the 3 months prior to screening
- Have recently changed their diet (less than 4 weeks)
- Have not been on a stable therapy with DMARDs in the 3 months prior to enrolment
- Experienced any gastrointestinal symptom (diarrhoea, vomiting, abdominal pain, constipation) in the 4 weeks prior enrolment
- Taken antibiotics in the 3 months prior to enrolment
- Have been diagnosed with cancer in the past 3 years or any primary or secondary immunodeficiency
- Have had endoscopy procedures carried out in the 8 weeks prior to enrolment or any gastro-intestinal surgery in the last 6 months
- Make regular use of laxatives and/or agents that affect gut motility

The healthy volunteers will be approached by the healthy volunteer data manager at Imperial College London and will also able to self-refer through advertisements. After consenting there will be a questionnaire. The volunteer will be asked to complete a self-

Table 2 Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT)

	Mi-PART STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT(weeks)	***-2***	***-1***	***0***	***12***	***24***	***T_{24+} 12 months***
ENROLMENT						
Eligibility screen	X					
Informed consent	X					
Clinical assessments		X				
Allocation		X				
ASSESSMENTS						
Baseline variables	X	X	X	X	X	
Outcome variables			X	X	X	X
Data process variables				X	X	X

description of health. This will be evaluated by one of the investigators, and any healthy volunteer with no current medical conditions and not in use of prescribed or self-prescribed medication (except for multivitamins) will be enrolled in the study.

All the participants to the study will receive a physical examination with assessment of vital signs (pulse and blood pressure), weight, height and body mass index (BMI). The medical and medication history will be reviewed with collection of data on demographics, diet and lifestyle. All concomitant medications will be recorded, including probiotics or fermented milk products.

Patients with AS will be evaluated with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the Bath Ankylosing Spondylitis Functional Index (BAFMI) and the Bath Ankylosing Spondylitis Metrology Index (BASMI). Patients with PsA will be evaluated following the CASPAR criteria, the Psoriasis Area and Severity Index (PASI) and the Composite Psoriatic Disease Activity Index (CPDAI). In cases where dactylitis or enthesitis are features, the Leeds Dactylitis Index (LDI) and the Leeds Enthesitis Index (LEI) will be included, respectively. BASDAI, BASMI and BAFMI will be used in cases of PsA with spondyloarthropathy.

All patients will have to complete a Health assessment questionnaire (HAQ), Visual Analogue Scoring (VAS) and a 7-day food diary. PsA patients will have also to complete a Dermatology Life Quality Index Questionnaire (DLQI).

Routine clinical laboratory tests will be performed on all patients other than healthy volunteers, including: full blood count (FBC), liver function tests (LFT), urea and electrolytes (U&E), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) (London site) / plasma viscosity (PV) (Bath site). Samples will be collected for analysis from all patients: serum, plasma, urine and faeces, as well as DNA assessment.

Withdrawal criteria

Study participants will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator can withdraw subjects from the study for any of the following grounds:

1. Subject request.
2. Subject is lost to follow-up (LTFU).
3. Development of an intercurrent illness, condition, or procedural complication, which would interfere with the subject's continued participation.
4. The investigator also reserves the right to withdraw subjects in the interest of subject safety and welfare.

Adverse events

Definition

Adverse Event (AE) Any untoward medical occurrence in a patient or clinical study subject [24]. This includes occurrences that are not necessarily caused by or related to a medicinal product administered. An adverse event can, therefore, be any unfavourable and unintended signs [25], abnormal laboratory values, and symptoms or disease temporarily associated with or not associated with study activities.

Serious Adverse Event (SAE) Serious Adverse Event. An untoward occurrence that: (a) results in death; (b) is life-threatening; (c) requires hospitalisation or prolongation of existing hospitalisation; (d) results in persistent or significant disability or incapacity; (e) consists of a congenital anomaly or birth defect.

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious [26].

Reporting procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

All such events, whether expected or not, should be recorded. This starts from the signed informed consent for participation in the trial until the last visit.

Adverse events should be documented in the participant's source document sheet.

Adverse events to be graded according to the degree of severity as follows:

Grade 1 - Mild, usually transient in nature and does not interfere with normal activity, observation only, no intervention, is needed.
Grade 2 - Moderate, interfere with normal activities minimal, local or non-invasive intervention indicated; limiting age-appropriate.
Grade 3 - Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL.
Grade 4 - Life-threatening consequences; urgent intervention indicated.
Grade 5 - Death related to AE.

Abnormal laboratory tests results constitute adverse events only if they:

1. Induce clinical signs and symptoms
2. They are considered clinically significant by the investigator
3. They require intervention

Clinically significant abnormal laboratory test results should be identified through a review of Lab values outside normal ranges as per hospital policy and procedures. Notable or significant changes from the baseline or previous visit values, which are considered to be clinically significant, to be documented in the medical notes and reported to their general practitioner and Serious AEs.

An SAE form should be completed and given/sent to the Chief Investigator within 24 h. However, relapse and death due to PsA or AS and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All SAEs should be reported to the approving REC where in the opinion of the Chief Investigator, the event was:

1. 'related',i.e. resulted from the administration of any of the research procedures; and
2. 'Unexpected', i.e. an event that is not an expected occurrence of the study procedures.

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Trial challenge

Emerging evidence has been linking the microbiome with several diseases including autoimmune diseases such as Psoriatic Arthritis [14]. In a paper published in 2015 [21], Scher and colleagues described the observation of a less diverse microbiome in 16 subjects affected by Psoriatic arthritis. Comparing samples taken from patients with psoriatic arthritis, psoriasis and healthy volunteers they observed a lower abundance of specific beneficial taxa of the gut microbiome in the first two groups. Furthermore, the patients with psoriatic arthritis showed other immunologic alterations with increase in fecal sIgA levels, decrease of RANKL and Osteoprogerin (which may affect the antigen presentation process in the gut) and decreased quantities of medium chain fatty acids (MCFAs). From studies conducted on healthy volunteers we know that the microbiome is constantly reshaping under the influence of individual and environmental factors [27–29]. Among the environmental factors, the diet [30, 31] exerts an extremely important role. It has been documented that a change of diet is actually able to modify the microbiome in as little as one or 2 days [32]. In order to detail accurately the relationship between food intake and microbiome, Johnson and colleagues [33] conducted a small clinical trial study on 34 healthy volunteers who were randomised to introduce with different diets. The results of the trial show the potential role of different food and micronutrients in influencing different bacteria strains in a population of healthy volunteers.

The hypothesis of our study was that the microbiome-metabolic interface of psoriatic arthritis patient is distinct comparing to healthy volunteers. The food diary included in the study enlists different elements in the 5 food groups and the possible effect of portion size is taken into account using visual aids to help the patients to describe in the most accurate way the amount of food they have introduced with any meal. The time of the different meals is taken into consideration as are soft and alcoholic drinks.

Sample size consideration

Estimate adequate sample sizes is complicated due to the lack of consensus in microbiome studies. Classical power calculations, used for clinical trials, are not a robust manner to assess the numbers of individuals required for exploratory and observational microbiome studies [34] since the SD and means of the sample populations is not known.

The recruitment strategy will be based on previous studies in inflammatory arthropaties [15, 21]. The variance in Th17, Treg numbers, IL-17 and IL – 22 cytokine measurements was estimated on the basis of previous clinical trials results [24]. Assuming a 2-sided significance level of 5% ($a = 0.05$) and a power 1-b of 95% (level at which we set the null hypothesis) and a 25% difference in IL – 17A secretion between controls and PsA, we needed to recruit a minimum of 24 study participants into each group (healthy volunteer, PsA and AS). Due to the nature of the sampling and the multiple visits to the clinic, we have set the target in 30 volunteers or more per group, to allow for dropout rate of 10% and to ensure robustness. First approach to subject lost to follow-up would be replaced to maximise data outcome and study objectives, otherwise the subject would be included with the acquired data.

In conclusion the study target to enrol 65 eligible patients with psoriatic arthritis (45 at the Bath centre and 20 at the London centre), 30 with ankylosing spondylitis, and 30 healthy volunteers.

Analysis plan and data management

The phenomic characterisation will take place during the later stage of the study (Table 2) and it involves 3 different arms of analysis: microbiome, immunology and metabonome analysis.

Bacterial DNA will be extracted from faecal samples, to define bacterial diversity and taxonomy. Whole genome sequencing, 16S rRNA gene sequencing and Nanopore sequencing will provide a taxonomic analysis and taxon list in PsA, AS and healthy individuals. Furthermore, this procedure will provide a comparative between the different sequencing technologies and its possibilities in clinical studies.

The immunological analysis will measure proportions of Th17and Treg subsets in peripheral blood samples using multi-parametric flow cytometry. T cells subsets will be use to look for associations between composition of gut microbiome and proportion of circulating Th17 and Treg in study participants. Serum samples will also be used for analysis of pro-inflammatory cytokines (IL-1β, IL-6, and IL-23p19).

NMR and UPLC-MS measurements of all urine, blood and faecal samples for metabolite profiling will be used for the metabonomic characterization. This metabonomic dataset will be used for biomarker ID and functional metabolic discriminators of PsA.

Finally, gut microbiome sequencing datasets will be integrated with biofluid metabolite profiles to identify important biological pathways in specific individuals. In the first stage we will correlate the metabolic profiles from NMR based metabonomics with the pattern of the abundance of microbial taxonomic entities and in a second phase evaluating enrichment of specific ontologies or metabolic functions from metagenomic databases. Once key pathways are statistically pinpointed, we will look into metabolic fluxes as integrated by different taxonomic entities.

The aim is to link gene composition, host metabotypes and immunotypes. We will use the whole gut microbiota community to pinpoint which functions are enriched/depleted in PsA cohorts and whether there are functions which correlate with disease or act as biomarkers of disease.

Meta-gene analysis (i.e. targeted functional metagenomics) will be used to establish the bile salt hydrolases, SCFA synthesizing genes and glucuronidases in gut samples. This approach will link specific functional gene diversity with specific metabotypes.

To ensure that all the data from the study will also be accessible, Mi-PART will upload all his dataset to The European Genome – Phenome Archive [35] and some specific data such as the taxonomy and species diversity of the study will be uploaded to NCBI Taxonomy [36]. Subsequently, data will be made available to the public in the main manuscript or additional supporting files in open access publications.

All data collected during the course of the study will be anonymised with either a temporary I.D. or enrolment I.D and stored at each site in a locked filing cabinet, with restricted access, and disposal arrangements of participant personal and clinical data. Shared data for analysis between centres will be anonymised, password locked and encrypted. Secure platform for sharing data will include email (nhs.net), and other secure media (encrypted USB and CD) via registered mail.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period. The study documentation for the external sites will be transferred and archived at the Imperial College Archives and Corporate Records Unit. Patient identifiable data (PID) will be stored at the respective sites.

Chief Investigator (CI) has a responsibility to ensure that participant anonymity is maintained and protected against any unauthorised parties. Information with regards to study participants will be kept confidential and managed in agreement with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval.

To ensure this is done accordingly, each participant at a time of consent will be allocated a unique screening number/code before undergoing any screening procedures. The study management team, together with all clinical site team, will comply with all aspects of the Data Protection Act 1998. All information collected will be kept confidential, and consent forms (containing participants' full names) will be held entirely separate to another data.

All data collection forms will be anonymised with either a temporary I.D. or enrolment I.D and stored at each site in a locked filing cabinet, with restricted access, and disposal arrangements of participant personal and clinical data.

The anonymised electronic data will be stored and shared on Imperial College Box file storage site. The electronic database used for this study is a bespoke system which uses a robust tried and tested security system with restricted access and routine backup protocols in place.

The anonymised, password locked encrypted data can also be shared between centres using secure email (nhs.net), and other secure media (encrypted USB and CD) via registered mail.

Participants will not be identifiable in the results of the study. The samples collected will also be anonymised with enrollment ID, cohort ID and study visit number. Samples will be kept in the tissue bank at the site of

collection until ready for analysis. Anonymised samples that require immediate processing and analysis will be shipped immediately to the designated centres at Imperial College London and Bath University.

Sample collected across sites for processing and analysis will be transferred via registered courier compliant to UN3373 biological substance category B shipment. The participants will be anonymised with regards to any future publications relating to this study.

At the end of the study residual samples will be transferred to a tissue bank.

Discussion

Growing evidence during recent years has linked gut microbiome alterations with an increased disposition to develop chronic rheumatic diseases [15]. Particularly, the PsA link to the microbiome has been the target of a number of promising studies [12, 37–40]. However, there is a paucity of information at the microbiota-metabolite-immunology network level in the setting and development of the disease.

Taking into consideration the gap between these early stages of the research and clinical applications or therapeutic benefits. MI-PART is a multicentre, prospective, and observational study that aims to reduce the gap, undertaking a deep phenomic analysis.

Mi-PART study design acknowledges the heterogeneous manifestations of rheumatic diseases, particularly PsA. Previously, this has led to inaccuracies in the recruitment of patients for clinical studies [41]. All the recruitments in our study fulfil CASPAR Criteria [42] which has been proven the most feasible, specific and sensible for PsA diagnosis [43]. Mi-PART ensures a relevant number of recruitments since it has access to the Axial Disease in Psoriatic Arthritis (ADIPSA) [9] which counts with 200 PsA patients and also 200 AS patients that will set a cohort to analyse the heterogeneous manifestations of PsA and possible overlapping between the two diseases.

Previous studies have reported the relevance of control populations that should be used for microbiome studies [44, 45]. This study will recruit, in the first place, relatives of the PsA patients recruited for the study in order to ensure a similar background. In any circumstance, healthy controls sex, age, ethnicity, etc. will match the features of the PsA cohort to minimise environmental variables, particularly sensible in microbiome studies. Diet of all the participants in the study will be assessed using a 24-h food recall questionnaire for the previous 7 days of every visit during the study.

Diet pivotal role [31, 46, 47] in this study accounts for its connection with gut microbiome dynamics and high plasticity. However, an even bigger challenge is to understand and reconstruct the gut microbiome variability across time, for that reason longitudinal studies are extremely important. To address this, a PsA group in the study will have three different time points with a 12-week separation.

Recent reviews and guidelines for microbiome studies emphasize the need for transition from metataxonomics (16S rRNA gene sequencing) to metagenomics (whole metagenomics shotgun sequencing) since it would allow species-level identification and functional analysis [48–50]. Mi-PART study will run both methodologies in all the samples for a comparison of the cost-effectiveness in clinical studies and potential therapeutic interventions. Furthermore, the study will also use third-generation sequencing using Nanopore sequencing [51] which will give a timely state of the art of the technology and its possibilities for clinical studies and trials.

Even when sequencing technological development has been fundamental in microbiome studies, the importance of the host response to the changes of the microbiome has introduced multi-omics and phenomic analysis as an integrative tool to reveal microbe interaction with the immune and metabolic system [52]. This study would implement a phenomic approach, this work package uniquely interfaces genetics, microbiota, metabolic and inflammatory networks to elucidate the mechanisms driving PsA pathology.

In conclusion, the MI-PART trial is a human microbiome study sets to a full characterization of PsA microbiome and its biomarkers/signalling molecules. The study design, recruitment and sample manipulation have been focus on previous challenges in microbiome and chronic rheumatoid diseases studies, in order to minimize possible confounders and lack of standardization in the study pipeline. Investigating the role of the microbiome in the development of PsA could deepen our understanding of the pathogenesis of the disease and potentially open the way to new therapies.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s41927-020-00155-2.

Additional file 1. Includes appendices with the different questionnaires use for groups A, B, C & D of the study.

Abbreviations

16S RNA: 16S ribosomal RNA; AS: Ankylosing Spondylitis; BAFMI: Bath Ankylosing Spondylitis Functional Index; BASDAI: Bath Ankylosing Spondylitis Activity index; BASMI: Bath Ankylosing Spondylitis Metrology Index; BMI: Body Mass Index; CASPAR: Criteria for Psoriatic Arthritis; CPDAI: Composite Psoriatic Disease Activity index; CRP: C-Reactive Protein; DLQI: Dermatology Life Quality Index Questionnaire; DMARDs: Disease Modifying Anti-Rheumatic Drugs; DNA: Deoxyribonucleic acid; ESR: Erythrocyte Sedimentation Rate; FBC: Full Blood Count; HAQ: Health assessment questionnaire; HLA-B27: Human Leucocyte Antigen B27; ICF: Informed Consent Form; IL13: Interleukin 13; IL17A: Interleukin 17A; IL22: Interleukin 22; LDI: Leeds Dactylitis Index; LEI: Leeds Enthesitis Index; LFCAs: Long Chain Fatty Acids; LFT: Liver Function Tests; MCFAs: Medium Chain Fatty Acids; PASI: Psoriasis Activity Score index; PID: Patient identifiable Data; PIS: Patient Information Sheet; PsA: Psoriatic Arthritic; PTPN22: Protein

Tyrosine Phosphatase Non-Receptor type 22; PV: Plasma Viscosity; qPCR: Quatitative polymerase chain reaction; RANKL: Receptor activator of nuclear factor kappa-B ligand; RNA: Ribonucleic acid; SCFAs: Small Chain Fatty Acids; sIgA: Secretive Immunoglobuline A; TH17: T helper cell 17; U&E: Urea and Electolytes; VAS: Visual Analogue Scoring

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Study registration
The Integrated Research Application System (IRAS) (reference) project ID is 217,745. The study ID for the Joint Research Compliance office is 17HH3903. The Joint Research Compliance Office (reference) role is to help Imperial College London and its researchers meet the requirements of research governance, ensuring Imperial fulfils the legal, ethical and scientific obligations of the healthcare research process.

Trial status
The recruitment began in December 2017 with the first participant recruited at the Imperial College site.
The recruitment is expected to continue until 2021.

Protocol version and date
The protocol currently in use is version V3.0 dated 05 June 2019.

Audits
The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

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Sponsor
Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:
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Authors' contributions
JMB and FB were responsible for conception, literature review, writing and revising the manuscript. JRM, SA, NM and PK conceived and developed the idea for the study. SA and NM are the principal investigators of the study. JRM is the study coordinator. SA, NM and PK sat up the inclusion and exclusion criteria for the study. JRM and JMB are responsible for the phenomic analysis. In conclusion, all participants designated as authors critically either drafted or revised the first draft of the study protocol and the protocol paper. Also, all authors have approved the final version before submission.

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Availability of data and materials
Not applicable.

Ethics approval and consent to participate
This trial was approved by the Research Ethics Committee of East Midlands – Leicester Central Research (REC reference number: 17/EM/0175). The ethics approval covers all centres where the study takes place.
Written consent must be obtained, utilising the appropriate consent, prior to any study specific procedures being performed, including any study specific screening procedures prior to enrolment (unless already taken as part of routine care e.g. routine bloods). At the time of consent, participants must be informed that they have the right to withdraw their participation in the trial, and also their samples, at any stage and that doing so will not prejudice their future clinical management and care.
The original consent forms will be filed in the Site Investigator File; a copy of the consent forms will be given to the participant, and one filed in the hospital notes. The written consent will be taken by either by the PI or by a clinician to whom that the PI has delegated responsibility. The process of obtaining written consent will be clearly documented in the participant's medical notes.
All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

Consent for publication
Anonymised study results will be presented at relevant conferences and symposiums as a means of early communication. Full reports and papers will then be prepared for publication in high impact medical journals. The reports and papers that are published about the research will not identify patients who participated in this study.

Competing interests
The authors declare that they have no competing interests.

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References

1. Ogdie A, Langan S, Love T, Haynes K, Shin D, Seminara N, et al. Prevalence and treatment patterns of psoriatic arthritis in the UK. Rheumatol (United Kingdom). 2013;52(3):568–75.
2. Merola JF, Li T, Li W-Q, Cho E, Qureshi AA. Prevalence of psoriasis phenotypes among men and women in the USA. Clin Exp Dermatol. 2016; 41(5):486–9 Available from: http://doi.wiley.com/10.1111/ced.12805. [cited 2020 May 18].
3. Walters JRF, Marchesi JR. Chronic diarrhea, bile acids, and Clostridia. J Clin Invest. 2019;130(1) Available from: https://www.jci.org/articles/view/133117. [cited 2019 Dec 12].
4. Veale DJ, Fearon U. The pathogenesis of psoriatic arthritis. Lancet. 2018; 391(10136):2273–84. Available from. https://doi.org/10.1016/S0140-6736(18)30830-4.
5. Kane D, Stafford L, Bresniham B, FitzGerard O. A prospective, clinical and radiological study of early psoriatic arthritis: an early synovitis clinic experience. Rheumatology. 2003;42(12):1460–8.
6. Chandran V, Schentag CT, Brockbank JE, Pellett FJ, Shanmugarajah S, Toloza SMA, et al. Familial aggregation of psoriatic arthritis. Ann Rheum Dis. 2009; 68(5):664–7.
7. Furst DE, Belasco J, Louie JS. Genetic and inflammatory factors associated with psoriatic arthritis: Relevance to diagnosis and management. Clin Immunol. 2019; Available from: https://www.sciencedirect.com/science/article/pii/S152166161830679X?via%3Dihub. [cited 2019 Feb 18].
8. Queiro R, Morante I, Cabezas I, Acasuso B. HLA-B27 and psoriatic disease: a modern view of an old relationship. Rheumatol (United Kingdom). 2015; 55(2):221–9.
9. Jadon DR, Sengupta R, Nightingale A, Lindsay M, Korendowych E, Robinson G, et al. Axial Disease in Psoriatic Arthritis study: defining the clinical and radiographic phenotype of psoriatic spondyloarthritis. Ann Rheum Dis. 2017; 76(4):701–7 Available from: http://www.ncbi.nlm.nih.gov/pubmed/27913376. [cited 2020 May 13].
10. Love TJ, Zhu Y, Zhang Y, Wall-Burns L, Ogdie A, Gelfand JM, et al. Obesity and the risk of psoriatic arthritis: a population-based study. Ann Rheum Dis. 2012;71(8):1273 Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3645859/. [cited 2020 May 20].
11. Li W, Han J, Qureshi AA. Smoking and risk of incident psoriatic arthritis in US women. Ann Rheum Dis. 2012;71(6):804–8 Available from: https://ard.bmj.com/content/71/6/804.long. [cited 2020 May 20].
12. Hsu C-Y, Kuo H-C, Su Y-J. Gut microbiota differences between psoriatic arthritis and undifferentiated arthritis patients. Res Sq. 2020. p. 1–19. https://doi.org/10.21203/rs.3.rs-15400/v1.
13. Myers B, Brownstone N, Reddy V, Chan S, Thibodeaux Q, Truong A, et al. Best Practice & Research Clinical Rheumatology the gut microbiome in psoriasis and psoriatic arthritis. Best Pract Res Clin Rheumatol. 2020;(xxxx): 101494. Available from. https://doi.org/10.1016/j.berh.2020.101494.
14. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system the gut microbiota interactions between the microbiota and the immune system. Science. 2012;336(6086): 1268–73 Available from: http://www.sciencemag.org/content/336/6086/1268.full.html%5Cn. http://www.sciencemag.org/content/336/6086/1268.full.html#related%5Cn. http://www.sciencemag.org/content/336/6086/1268.full.html#ref-list-1%5Cn. http://www.sciencemag.org/cgi/collection/immunology.
15. Salem F, Kindt N, Marchesi JR, Netter P, Lopez A, Kokten T, et al. Gut microbiome in chronic rheumatic and inflammatory bowel diseases: Similarities and differences. United Eur Gastroenterol J. 2019;7(8):1008–32 Available from: http://journals.sagepub.com/doi/10.1177/2050640619867555. [cited 2019 Oct 31].
16. Naydich AD, Nangle SN, Bues JJ, Trivedi D, Nissar N, Inniss MC, et al. Synthetic gene circuits enable systems-level biosensor trigger discovery at the host-microbe interface. mSystems. 2019;4(4):e00125–19 Available from: https://msystems.asm.org/content/4/4/e00125-19. [cited 2019 Jun 21].
17. Martinez KA, Devlin JC, Lacher CR, Yin Y, Cai Y, Wang J, et al. Increased weight gain by C-section: Functional significance of the primordial microbiome. Sci Adv. 2017;3(10):eaao1874 Available from: https://advances.sciencemag.org/lookup/doi/10.1126/sciadv.aao1874. [cited 2020 May 20].
18. Meng X, Zhou H-Y, Shen H-H, Lufumpa E, Li X-M, Guo B, et al. Microbe-metabolite-host axis, two-way action in the pathogenesis and treatment of human autoimmunity. Autoimmun Rev. 2019;18(5):455–75 Available from: https://www.sciencedirect.com/science/article/pii/S156899721930059X. [cited 2019 Jun 6].
19. Manasson J, Scher JU. Spondyloarthritis and the microbiome: new insights from an ancient hypothesis. Curr Rheumatol Rep. 2015;17:10. https://doi.org/10.1007/s11926-014-0487-7.
20. Taurog JD, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernández-Sueiro JL, et al. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. J Exp Med. 1994;180(6): 2359–64 Available from: http://www.ncbi.nlm.nih.gov/pubmed/7964509. [cited 2019 Jul 2].
21. Scher JU, Ubeda C, Artacho A, Attur M, Isaac S, Reddy SM, et al. Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. Arthritis Rheumatol (Hoboken, NJ). 2015;67(1):128–39 Available from: http://www.ncbi.nlm.nih.gov/pubmed/25319745.
22. Sonner JK, Keil M, Falk-Paulsen M, Mishra N, Rehman A, Kramer M, et al. Dietary tryptophan links encephalogenicity of autoreactive T cells with gut microbial ecology. Nat Commun. 2019;10(1):4877 Available from: http://www.nature.com/articles/s41467-019-12776-4. [cited 2019 Oct 30].
23. Department of Health. Research governance framework for health and social care. Health Soc Care Community. 2005;10(1):1–54.
24. Golder S, Loke YK, Wright K, Norman G. Reporting of adverse events in published and unpublished studies of health care interventions: A systematic review. Ioannidis JP, editor. PLOS Med. 2016;13(9):e1002127 Available from: https://dx.plos.org/10.1371/journal.pmed.1002127. [cited 2020 May 24].
25. Lagging M, Brown A, Mantry PS, Ramji A, Weilert F, Vierling JM, et al. Grazoprevir plus peginterferon and ribavirin in treatment- naive patients with hepatitis C virus genotype 1 infection : a randomized trial. J Viral Hepat. 2016;23:80–8.
26. Eakin JM. Reframing the evaluation of qualitative health research: reflections on a review of appraisal guidelines in the health sciences. J Eval Clin Pract. 2002; Available from: https://pdfs.semanticscholar.org/19b2/4b20438b246d527fff51726885faca0200fe.pdf. [cited 2020 May 24].
27. Buford TW. (Dis)Trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome. 2017;5(1):80 Available from: http://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-017-0296-0. [cited 2020 May 24].
28. Audet MC. Stress-induced disturbances along the gut microbiota-immune-brain axis and implications for mental health: Does sex matter? Front Neuroendocrinol. 2019;54 Available from: https://pubmed.ncbi.nlm.nih.gov/31302116/. [cited 2020 May 24].
29. Capurso G, Lahner E. The interaction between smoking, alcohol and the gut microbiome. Best Pract Res Clin Gastroenterol. 2017;31(5) Available from: https://pubmed.ncbi.nlm.nih.gov/29195678/. [cited 2020 May 24].
30. Singh RK, Chang H-W, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med. 2017;15:73 Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5385025/pdf/12967_2017_Article_1175.pdf. [cited 2019 Jul 23].
31. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559–63 Available from: http://www.nature.com/articles/nature12820. [cited 2019 Jul 12].
32. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559–63 Available from: http://www.ncbi.nlm.nih.gov/pubmed/24336217. [cited 2019 Jul 12].
33. Johnson AJ, Vangay P, Al-Ghalith GA, Menon R, Koecher K, Knights CD. Daily sampling reveals personalized diet-microbiome associations in humans. Cell Host Microbe. 2019;25:789–802.e5 Available from: https://doi.org/10.1016/j.chom.2019.05.005. [cited 2019 Jul 2].
34. La Rosa PS, Brooks JP, Deych E, Boone EL, Edwards DJ, et al. Hypothesis testing and power calculations for taxonomic-based human microbiome data. PLoS ONE. 2012;7(12):e52078. https://doi.org/10.1371/journal.pone.0052078.
35. EGA European Genome-Phenome Archive. Available from: https://ega-archive.org/. [cited 2020 Jun 23].

36. Taxonomy - NCBI. Available from: https://www.ncbi.nlm.nih.gov/taxonomy. [cited 2020 Jun 23].
37. Dumas E, Venken K, Rosenbaum JT, Elewaut D. Intestinal microbiota, HLA-B27, and spondyloarthritis: dangerous liaisons. Rheum Dis Clin N Am. 2020; 46(2):213–24.
38. Wen C, Zheng Z, Shao T, Liu L, Xie Z, Le Chatelier E, et al. Quantitative metagenomics reveals unique gut microbiome biomarkers in ankylosing spondylitis. Genome Biol. 2017;18(1):1–13.
39. Rosser EC, Piper CJM, Matei DE, Wedderburn LR, Eaton S, Mauri C, et al. Microbiota-derived metabolites suppress arthritis by amplifying aryl-hydrocarbon receptor activation in regulatory B cells article microbiota-derived metabolites suppress arthritis by amplifying aryl-hydrocarbon receptor activation in regulatory B cells. Cell Metab. 2020;31:1–15.
40. Eppinga H, Thio HB, Peppelenbosch MP, Konstantinov SR. The gut microbiome dysbiosis and its potential role in psoriatic arthritis. Int J Clin Rheumtol. 2014;9(6):559–65.
41. Manasson J, Blank RB, Scher JU. The microbiome in rheumatology : Where are we and where should we go ? Ann Rheum Dis. 2020;79:1–7.
42. Tillett W, Costa L, Jadon D, Wallis D, Cavill C, McHugh J, et al. The ClASsification for Psoriatic ARthritis (CASPAR) criteria--a retrospective feasibility, sensitivity, and specificity study. J Rheumatol. 2012;39(1):154–6 Available from: http://www.ncbi.nlm.nih.gov/pubmed/16463433. [cited 2019 Mar 20].
43. Mohamad Ali Rida VC. Challenges in the clinical diagnosis of psoriatic arthritis. Clin Immunol. 2020;111554. Available from. https://doi.org/10.1016/j.molliq.2019.111554.
44. Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc Natl Acad Sci U S A. 2011;108(Suppl 1) Available from: https://pubmed.ncbi.nlm.nih.gov/20571116/. [cited 2020 May 24].
45. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, et al. Environment dominates over host genetics in shaping human gut microbiota. Nature. 2018;555(7695):210–5 Available from: http://www.nature.com/articles/nature25973. [cited 2020 May 24].
46. Johnson AJ, Vangay P, Al-Ghalith GA, Hillmann BM, Ward TL, Shields-Cutler RR, et al. Daily Sampling Reveals Personalized Diet-Microbiome Associations in Humans. Cell Host Microbe. 2019;25(6) Available from: https://pubmed.ncbi.nlm.nih.gov/31194939/. [cited 2020 May 24].
47. Onyszkiewicz M, Jaworska K, Ufnal M. Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system. Exp Biol Med. 2020;153537021990089 Available from: http://journals.sagepub.com/doi/10.1177/1535370219900898. [cited 2020 Jan 22].
48. Tsou AM, Olesen SW, Alm EJ, Snapper SB, Tsou AM, Olesen SW, et al. 16S rRNA sequencing analysis : the devil is in the details 16S rRNA sequencing analysis : the devil is in the details. Gut Microbes. 2020;00(00):1–4. Available from. https://doi.org/10.1080/19490976.2020.1747336.
49. Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD, et al. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. Nature. 2016;533(7604):543–6 Available from: http://www.nature.com/articles/nature17645. [cited 2020 May 24].
50. Donlin LT, Park S-H, Giannopoulou E, Ivovic A, Park-Min K-H, Siegel RM, et al. Insights into rheumatic diseases from next-generation sequencing. Nat Rev Rheumatol. 2019;1 Available from: http://www.nature.com/articles/s41584-019-0217-7. [cited 2019 Apr 29].
51. Moss EL, Maghini DG, Bhatt AS. Complete, closed bacterial genomes from microbiomes using nanopore sequencing. Nat Biotechnol. 2020:1–7 Available from: http://www.nature.com/articles/s41587-020-0422-6. [cited 2020 Feb 21].
52. Knight R, Navas J, Quinn RA, Sanders JG, Zhu Q. Best practices for analysing microbiomes. Nat Rev Microbiol. 2018; Available from. https://doi.org/10.1038/s41579-018-0029-9.

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