Contemporary Clinical Trials Communications 7 (2017) 158–162

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: www.elsevier.com/locate/conctc

Successful collection of stool samples for microbiome analyses from a large community-based population of elderly men

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ARTICLE INFO

Keywords:
Microbiome
Aging
Men
Gastrointestinal
Genotyping
Stool

ABSTRACT

The relationship of the gastrointestinal microbiome to health and disease is of major research interest, including the effects of the gut microbiota on age related conditions. Here we report on the outcome of a project to collect stool samples on a large number of community dwelling elderly men using the OMNIgene-GUT stool/feces collection kit (OMR-200, DNA Genotek, Ottawa, Canada). Among 1328 men who were eligible for stool collection, 982 (74%) agreed to participate and 951 submitted samples. The collection process was reported to be acceptable, almost all samples obtained were adequate, the process of sample handling by mail was uniformly successful. The DNA obtained provided excellent results in microbiome analyses, yielding an abundance of species and a diversity of taxa as would be predicted. Our results suggest that population studies of older participants involving remote stool sample collection are feasible. These approaches would allow large scale research projects of the association of the gut microbiota with important clinical outcomes.

1. Introduction

The human microbiome is the composition of microorganisms (e.g. bacteria, virus, fungi, and parasites) and microbial products that inhabits the human body. The microbes in a healthy human adult are estimated to at least equal the number of human cells [1]. It has been long known that bacteria are involved in certain body processes, such as digesting food and producing vitamins, but the microbiome may have a much broader impact on our health than was previously realized. The community of microbes in an individual may influence the susceptibility to certain infectious diseases, as well as contribute to disorders such as obesity [2], diabetes [3], and some chronic illnesses of the gastrointestinal system such as Crohn's disease and irritable bowel syndrome [4].

The influence of the gastrointestinal microbiome on health and disease is of major research interest. Stool specimens offer the most accessible means of assessing the gut microbial community, and the number of reports concerning the association of stool microbiome with physiological and medical conditions is increasing quickly. Nevertheless, there are few data concerning practical methods for collecting stool specimens for microbiome analyses. This is particularly true in the context of large, population-based studies that demand successful recruitment approaches, acceptable specimen collection methods, and successful transport and processing of large numbers of samples. These issues may be especially challenging in the study of elderly populations in which remote sample collection is imperative.

Here we report the results of an effort to collect stool samples from a large number of older men enrolled in the Osteoporotic Fractures in Men Study (MrOS). Using convenient stool collection methods, we developed a successful approach for remote sample acquisition and preservation that could be applied to similar population-based research.

Abbreviations: CMMR, Alkek Center for Metagenomics and Microbiome Research; DNA, Deoxyribonucleic Acid; DXA, Dual Energy X-ray Absorptiometry; FFQ, Food Frequency Questionnaire; HRpQCT, High Resolution Peripheral Quantitative Computed Tomography; MrOS, Osteoporotic Fractures in Men Study; NCATS, National Center for Advancing Translational Sciences; NIA, National Institute on Aging; NIAMS, National Institute of Arthritis and Musculoskeletal and Skin Diseases; OTU, Operational Taxonomic Units; rDNA, Recombinant Deoxyribonucleic Acid; SILVA database, a comprehensive on-line resource for quality checked and aligned ribosomal RNA sequence data; UPARSE, cluster algorithm; USEARCH, search and cluster algorithm

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http://dx.doi.org/10.1016/j.conctc.2017.07.002
Received 30 January 2017; Received in revised form 28 June 2017; Accepted 5 July 2017
Available online 06 July 2017

2. Materials and methods

2.1. Subjects and overall MrOS study protocol

MrOS study is a prospective study of 5994 older men, recruited at six clinical U.S. sites between 2000 and 2002. The cohort and recruitment methods have been previously described [5]. At baseline, participants were at least 65 years of age, able to consent, walked without assistance of another person, and did not have bi-lateral hip replacement or any condition that in the judgment of the site investigator that would likely impair participation in the study [6]. The Institutional Review Boards at all sites reviewed and approved the study and all participants provided informed consent.

Since its inception and baseline examination, participants have returned for at least 4 full in-clinic examinations, completed tri-annual postcards concerning outcomes of interest, and completed two extensive interim questionnaires concerning lifestyle and medical issues. The rate of ongoing participation in the MrOS study has been excellent. Of surviving participants, 99.4% had completed all study visits and 88.0% completed all tri-annual postcards used for regular follow-up.

In May 2014, men began returning for a fourth clinic visit that was comprehensive and complex. Participants completed a health history questionnaire at home, obtained objective activity monitoring by wearing accelerometry equipment for 7 days, and completed a variety of measures during a research clinic visit at one of the six participating institutions. Clinic measures included whole body, hip and spine dual energy x-ray absorptiometry (DXA), high resolution peripheral quantitative computed tomography (HRpQCT), measures of physical performance (short physical performance battery; 400 m walk, force plate for lower extremity power), measures of height and weight, blood and urine specimen collection, questionnaires and interviews. Participants were asked to rate their overall health in relationships to their similarly-aged peers (excellent, good, fair, poor). The visit took approximately 4–5 h to complete. The goal was to obtain these measures on 1950 men (70% of the surviving cohort).

2.2. Stool collection protocol

In March–April 2015, all six institutions began the collection of stool specimens for microbiome analyses. To recruit men for stool sample collection, study staff asked subjects at the research clinic visit if they would agree to provide a stool specimen to study the microbiome. For those who agreed, staff demonstrated the process with the collection materials in hand. Participants then took home a collection kit that included a toilet hat, instructions for collecting/mailing the specimen, a collection tube, exam gloves, alcohol wipes, a postage-paid return mailing envelope, and biohazard mailing bag. A short questionnaire about the collection time and date was included in the collection kit. A dietary recall questionnaire (Block 98.2 food frequency questionnaire (FFQ); modified for MrOS to capture the most frequently consumed sources of calcium, vitamin D, and other selected nutrients influencing risk of osteoporosis and prostate cancer in US men (Nutritionquest, Berkeley CA)) was completed by the participant at home and returned with the stool specimen.

Participants at all 6 MrOS sites mailed the samples directly to the Portland site for initial processing. Immediately upon receipt, study staff opened the packages to check for stool sample adequacy and to ensure the paperwork (time and date of collection) had been completed. Samples were then stored in a −80 °C freezer. If after two weeks an expected stool sample had not arrived at the central lab, the participant's study site was notified and a follow up phone call was made to ensure a sample was collected.

The participants from the Portland site completed an additional short questionnaire regarding the tolerability of the collection, time required and any perceived complications. This was mailed to the clinic with the specimen.

2.3. Kit for stool sample collection and preservation

The OMNIgene-GUT stool/feces collection kit (OMR-200, DNA Genotek, Ottawa, Canada) was used to collect stool samples. The OMNIgene-GUT collection kit is designed for the self-collection of a consistent volume of stool and preservation of microbial DNA. The tube includes a non-toxic stabilizing reagent and mixing apparatus and is safe for home use; personal protective equipment (e.g. gloves, eyewear, etc.) is not required. After the sample is collected and the tube is capped, the user vigorously shakes the tube for 30 s to homogenize and liquefy the sample. At that point the stool DNA is preserved for at least 60 days at ambient temperature [7,8]. The collection process has been successfully used in other clinical studies [9,10].

2.4. Bacterial genotyping

Six hundred specimens from unique participants were sent to the Alkek Center for Metagenomics and Microbiome Research (CMMR) at Baylor College of Medicine in Houston, Texas for microbiome analysis. Samples were arrayed in boxes and shipped on dry ice over-night with an accompanying sample manifest that included de-identified sample IDs and box positions. Upon delivery, samples were reconciled with the provided manifest and stored at −80 °C until further processing. For bacterial genomic DNA extraction, samples were thawed at room temperature to re-liquefy the samples and 200 μL of stool suspension were transferred to the extraction deep-well plate. For samples where the fecal material was too thick to pipette, an equivalent volume was transferred using a sterile and disposable spatula. DNA extraction was carried out in the Hamilton STARlet platform following the standard MoBio PowerMag Soil DNA extraction protocol. Extracted DNA was subjected to 16S (v4) rDNA amplification using primers 515F and 806R containing Illumina adapters and a single-end barcode allowing pooling and direct sequencing of PCR products [11]. Amplicons were visualized via gel electrophoresis and quantified via automated Quant-iT PicoGeen assay. Quantified amplicons were normalized and pooled according at DNA mass of 100 ng per sample, and the resulting amplicon pool was cleaned using the ChargeSwitch PCR Clean-up Kit (Invitrogen). The amplicon pool was sequenced on two lanes of an Illumina MiSeq reagent kit v2 (2 × 250 bp) and resulting sequences were demultiplexed based on the unique molecular barcodes, and reads were merged using USEARCH v7.0.1090 [12], allowing zero mismatches and a minimum overlap of 50 bases. Merged reads were trimmed at first base with Q5. In addition, a quality filter was applied to the resulting merged reads and reads containing above 0.05 expected errors were discarded.

The analytic pipeline for 16S rDNA analysis leverages custom analytic packages and pipelines developed at the CMMR to provide summary statistics and quality control measurements for each sequencing run, as well as multi-run reports and data-merging capabilities for validating built-in controls (known and blank) and characterizing microbial communities across large numbers of samples or sample groups.

16Sv4 rDNA gene sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm [13]. OTUs were mapped to an optimized version of the SILVA Database [14] containing only the 16Sv4 region to determine taxonomies. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. A custom script constructed a rarefied OTU table from the output files generated in the previous two steps for downstream analyses of alpha-diversity, beta-diversity [15] and phylogenetic trends.

3. Results

The mean age of the men at the time of their visit was 85.0 ± 4.5 years. After the stool collection process began 1328 men completed Visit 4 and 982 (74%) agreed to collect a stool specimen. Three hundred and forty-six (26%) men refused or were deemed ineligible to collect