Local Protocol #: HUM00082721

Characterizing Human Colonic and Stool Microbiome (KWS study)

INVESTIGATORS

D. Kim Turgeon, MD[1] (P.I.)
Mack T Ruffin, MD MPH[2]
Patrick Schloss, PhD[3]
Ananda Sen, PhD [2, 4]

[1]Division of Gastroenterology, Department of Internal Medicine
3912 Taubman Center
University of Michigan Medical Center
Ann Arbor, MI 48109
Tel (734) 764-6860
Fax (734) 764-2566

[2]Department of Family Medicine, University of Michigan

[3]Department of Microbiology and Immunology, University of Michigan

[4]Department of Biostatistics, University of Michigan

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SCHEMA

20 Healthy volunteers > 25years old

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Baseline Visit

- Consent for samples
- Data collection
- Stool kit provided (bucket, sampling vials, 2 FIT tests)
- Schedule colon procedure

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Un-prepped Colonoscopy with conscious sedation in MCRU

- Consent for colonoscopy/sedation (Physician only)
- Flexible Sigmoidoscopy for stool sample (RNA later)
- colonic mucosal biopsies from sigmoid colon (RNA later)

- Change endoscope to Colonoscope go to right colon (ideally just above ICV and take stool sample and colonic mucosal biopsies from right colon (RNA later)

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Recover patient in MCRU, discharge patient home with driver

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Pay subject and driver for participation

Abbreviations

University of Michigan Health System	UMHS
Michigan Clinical Research Unit	MCRU
Standard Operating Procedures	SOPs
Clinical Research Associate	CRA
Data Safety Monitoring Committee	DSMC
Institutional Review Board	IRB
Principal Investigator	PI
Adverse event	AE
Serious Adverse Event	SAE
Case report Form	CRF
Human subjects incentive program	HSIP
Fecal Immunohistochemical test	FIT
Primary Care Provider	PCP

1.0 BACKGROUND AND RATIONALE

Colorectal cancer (CRC) is the third most common cancer in the US, with approximately 150,000 new cases diagnosed every year[1]. CRC remains a significant burden in the United States [1] even in the setting of multiple effective screening options [2] and increased screening of adults age 50-75 years [3]. 33-47% of CRCs originate in the right side of the colon and 53-66% in the left side [4, 5]. One hypothesis suggests that these cancers have different etiologies with those from the left side driven by inflammation and those from the right side developing from genetics and the effects of toxins [6, 7]. One unexplored mechanism has been the role of the microbiome in these forms of CRC. A microbial etiology contribution to these two forms of CRC is plausible given the significant amount of microbial biomass in the colon and numerous links between the microbiome and driving tumorigenesis in the colon (e.g. production of toxins, inducing inflammation, and affecting angiogenesis and cell proliferation) [8-15]. Furthermore, a growing number of small cohort studies (N<100) have observed differences in the fecal microbiome between healthy individuals and those with CRC [9, 16-19]. Although there are a growing number of studies analyzing fecal material, there is a need to better understand the changes in the mucosa-associated microbiome. The etiological role of the microbiome in left and right-sided cancers has been ignored. Finally, it is accepted that the common bowel preparation for colonoscopy significantly alters the composition of the gut microbiome and so it would be preferred to characterize the mucosal surfaces in un-prepped colons, prior endoscopic microbiome studies have only been done on prepped colons.

2.0 STUDY OBJECTIVES

The overall aim of this research project is to determine the microbiome composition and diversity in right and left colon, in the mucosal surface of the right and left colon in healthy subjects as compared to spontaneously evacuated stool.

2.1 Objectives

I. The luminal microbiome composition and the diversity are different in the right and left colon.
II. The mucosal microbiome composition and the diversity are not different from the stool microbiome composition and diversity in the right and left colon.
III. A spontaneously evacuated stool microbiome composition and the diversity reflects the mucosal or stool microbiome in the left colon more than the right colon.
IV. A FIT collection sample is adequate for characterizing the microbiome composition and diversity.
V. Both the FIT test and the microbiome characterization can be run on one FIT sample.

3.0 ELIGBILITY CRITERIA

3.1 Inclusion Criteria

1. Healthy subjects 25 years or older.
2. Willing and able to sign informed consent

3. Women of child-bearing potential must have a negative pregnancy test on the day of the procedure or be post-menopausal. Post-menopausal women are defined as post-hysterectomy, or over 40 and at least 18 months without menses and not on birth-control.
4. Has an adult who can drive and escort the participant home or meets criteria for unsedated colonoscopy.

4.1 Unsedated colonoscopy criteria: If a subject has had a prior successful unsedated colonoscopy and desires the study colonoscopy to be performed without conscious sedation Dr. Turgeon would review the subject's record and make a determination on the suitability of this request. If she agrees that an unsedated procedure is appropriate then the subject does not need an escort.

3.2 Exclusion Criteria

1. Use of Aspirin or NSAID use within 7 days
2. Antibiotic use within 3 months
3. Current anticoagulant or anti platelet agent use
4. Subjects with known allergy or negative reaction to Fentanyl, Versed or Benadryl
5. No history of colon diseases (inflammatory bowel disease)
6. No history of abdominal surgeries
7. No use of oral steroids
8. No significant respiratory, liver, kidney or brain impairment
9. No Diabetes
10. Subjects on active chemotherapy or radiation treatment
11. Pregnant or trying to conceive

4.0 STUDY PLAN/ACCRUAL

4.1 Summary of Recruitment Plan

We intend to enroll up to 20 evaluable subjects. We expect to be able to enroll about 1 subject per week, so the study should take about 6 months to complete. Subjects will be recruited by advertisement, prior chemoprevention study interest, and UMClinicaltrials.org listings.

4.2 Baseline Visit

Subjects will be scheduled for a baseline visit with a clinical research associate (CRA). At this visit, the CRA will review the informed consent, eligibility, medical history and study procedures. If the subject agrees to participate they will sign the consent. The instructions for the stool collections, the stool collection kit, and procedures will be reviewed (Appendix A). Subjects will be instructed to send the stool sample back to UM at least one week before their scheduled colonoscopy. The colonoscopy procedure will be scheduled and the instructions for that procedure will be reviewed (Appendix B). If the subject is not willing to have the procedure, then they will not collect stool and not continue in the study.

4.3 Colonoscopy Visit

Subjects will come to MCRU for the procedure. Because of sedation, subjects will require someone else drive/escort them home. (If a subject has had a prior successful unsedated colonoscopy and desires the study colonoscopy to be performed without conscious sedation Dr. Turgeon would review the subject's record and make a determination on the suitability of this request. If she agrees that an unsedated procedure is appropriate then the subject does not need an escort,) The effects of sedation last several hours and standard discharge instructions in the MPU advise against operating heavy machinery, making important decisions, or going out. Subjects will come fasting since midnight, without prepping for the procedure. Subjects will be encouraged to have normal bowel movements, including just prior to the procedure if possible.

The unprepped colonoscopy will take place in MCRU. There is a room with a processor for endoscopic procedures. Because the final extent of the colonoscopy is planned to go to the right colon, it is necessary to provide conscious sedation for subject comfort. Conscious sedation (Fentanyl, Versed and Benadryl) are used routinely in colonoscopies. These agents are administered by a nurse under the direct supervision of a M.D. (Gastroenterologist). Anesthesiologists are not required to administer these agents at UM. As per both MCRU and UMHHC policy 62-11-001, we will have another physician (most likely Dr Ruffin), NPP (non-physician provider), or GI fellow in the room watching the cardiac monitor, O2 sat at vital signs while Dr. Turgeon performs the procedure and direct the conscious sedation.

A driver/escort must be present and in the waiting room of MCRU for the procedure to proceed. If a subject has had a prior successful unsedated colonoscopy and desires the study colonoscopy to be performed without conscious sedation Dr Turgeon would review the subject's record and make a determination on the suitability of this request. If she agrees that an unsedated procedure is appropriate then the subject does not need an escort. Prior to the colonoscopy, a physical exam will be performed by MCRU, including a urine pregnancy test if applicable. Vital signs will be checked as part of the physical exam.

Dr. D. Kim Turgeon is the physician who will perform the colonoscopies. She is a board-certified gastroenterologist who routinely performs endoscopy with conscious sedation at UMHS. The subject will be consented separately for the colonoscopy (using consent form in Appendix B) by Dr. Turgeon. Prior to the procedure an IV will be placed for access and the administration of sedatives and the line kept open with D5 ½ NS intravenous solution (whether or not sedation is planned). A Time out will be performed by the Dr. Turgeon, the MCRU nurse and CRAs. Dr. Turgeon will verify Mallampti status and heart and lung exam prior to the procedure as per MPU protocol. Sedation level and vital signs will be monitored by the MCRU nurse as well as a BLS (Basic Life Support) trained medical provider (as stated above per UMHHC policy 62-11-001) throughout the procedure.

The colonoscopy will proceed per UMHS (University of Michigan Health System) standard of care and guidelines for colonoscopy in the MCRU endoscopy suite. Dr. Turgeon who will be performing the procedures will evaluate and reevaluate (during the procedure) safety for the subject and proceed only if there are no concerns.

After conscious sedation the flexible sigmoidoscope will be inserted to about 25cm where stool samples will be obtained using the biopsy forceps or with an endoscopy brush.

Following the stool samples 6 biopsies (ideally as 2 bites each) will be obtained. These specimens will be the left colon specimens. The flexible sigmoidoscope will be withdrawn.

Following withdrawal of the flexible sigmoidoscope, a clean pediatric colonoscope will be inserted to the ascending colon (ideally within visualization of the ICV). This change in endoscopes is being done to avoid contamination of the channel with material from the left colon. If the ascending colon cannot be reached specimens will be obtained at the farthest location reached proximal to the splenic flexure. Photographic documentation of the most proximal area of the colon reached will be obtained. When the most proximal (right) colon is reached stool samples and biopsies will be obtain in a similar manner to the ones from the left with fresh forceps/brushes. The colonoscope will then be withdrawn.

Patient will be recovered in MCRU consistent with MPU standard of care. Subjects will be discharged home with a driver/escort. Subjects will be encouraged to call the study team for any issues, questions or concerns. They will be given post-procedure instructions and contact information. If no sedation is used the subject will have post procedure vital signs and remain in recovery on the unit for at least 10 minutes prior to discharge.

4.4 Payment to subjects

Subjects will receive $50 for sending their stool in properly (replacements may be obtained if needed). Subjects will receive $150 after the colonoscopy. The driver will receive $25 (if there is no escort/driver because sedation has not been given then this will not be paid. Parking passes will be provided for the CVC parking area. Payments will be provided via the UM HSIP (Human subjects incentive program) as a card or check.

4.5 Schedule of Events

Evaluation/ Procedure	Initial MCRU visit	Baseline Stool Sample (1-2 weeks before procedure)	Procedure Day
Informed Consent	X		
Assess Eligibility	X		
Medical History	X		
Stool Collection/FIT samples		X	
Physical Exam			X
Procedure/procedure consent			X
Urine pregnancy Test*			X
Vital Signs/ Height and Weight	X		X
Adverse Events			X (chart review)

*Post-menopausal women are defined as post-hysterectomy, or over 40 and at least 18 months without menses and not on birth-control.

5.0 SPECIMENS/SAMPLE COLLECTION

5.1 Stool Collection Kit

Subjects will be given a stool collection kit with instructions (Appendix A). The kit consists of a collection bucket, sampling vials, 2 Fecal Immunohistochemical tests (FITs), preservative buffer and a shipping box. The subject will be provided with a prepaid UPS shipping label or may deliver the stool box to the study team.

The whole stool sample (after sampling for the FIT tests and stool without preservative) will be preserved in a proprietary EDTA-containing buffer and will be shipped by the subject (pre-paid UPS labeled mailing kit identified by subject number only) directly to the Central Chemoprevention Laboratory at the University of Michigan. Stool samples will be processed according to Exact Sciences SOPs. Aliquots of homogenate will be frozen at –70° C or colder for batch shipment to the Schloss Laboratory. Whole stools will be homogenized in the Exact Buffer solution. A median size 120 gm stool yields about 840 ml of homogenate or twenty 35 ml homogenate aliquots. Each 35 ml homogenate aliquot will contain stool equivalents to 8 gm of stool.

FIT tests will be analyzed will be analyzed at the Chemoprevention Lab at the UM using analytic equipment provided by Polymedco, Inc. (OC-Auto) following standard operating procedures provided with the analyzer. The used FIT and the unused FIT will be frozen at –70° C or colder for batch shipment to the Schloss Laboratory.

5.2 Colonoscopy stool sample collection

Stool samples will be obtained with biopsy forceps or brush to obtain a suitable quantity as described above. The stool will be placed in RNA later and provided on ice to the Schloss laboratory immediately following the procedure.

5.3 Biopsy Collection

Biopsies will be placed in vials in RNA later and sent on ice to the Schloss lab immediately following the procedure. The samples from the right and left colon will be kept separate as described above.

5.4 Sample labeling/Specimen storage and handling

All samples will be labeled unique IDs that are not easily linked back to the subject. Subjects will have a unique code assigned as their participant ID and all specimens and kits will have codes that link to the PID. The analytic labs will not know the name or other identifying information about the subjects.

After processing all specimen will be stored in the Schloss Laboratory until they are used up. If future use is denied or withdrawn by the subject, best efforts will be made to stop any additional studies/testing and destroy any remaining specimens.

There are no outside of University of Michigan collaborators. These specimens will not be made available if requested by outside laboratories. There is no therapeutic or commercial value to these specimens. The RNA being analyzed is bacterial not human.

5.5 Assay Methodology

All 16S rRNA gene sequencing and processing will be done within the Schloss Laboratory housed in the Department of Microbiology & Immunology. Microbial genomic DNA will be extracted using the PowerSoil-htp 96 Well Soil DNA isolation kit (Mo Bio Laboratories) using an EPMotion 5075 pipetting system. The V4 region of the 16S rRNA gene from each sample will be amplified and sequenced using the Illumina MiSeq Personal Sequencing platform using protocols published by their laboratory [20]. Sequences will be curated as described previously using the mothur software package [21]. Parallel sequencing of a mock community will enable us to calculate the observed error rate, which is generally below 0.01%. All fastq sequence files and the MIMARKS-compliant metadata spreadsheet will be made publicly available through the Schloss laboratory's server and on NCBI's Short Read Archive [22]. The output from this stage of the microbiome analysis will be a table that indicates the relative abundance of each bacterial taxa observed in each community. This overall strategy, from sequencing to analysis, has been employed in a number of recent studies from the Schloss laboratory [23-25].

6.0 Potential Risks AND BENEFITS

6.1 Risks

Subjects may experience increased discomfort due to procedure. The risks of the research only portion of the procedures are the same as the risks of the endoscopy procedure alone.

These risks include:

- Aspiration (pneumonia) (<1%)
- GI Bleeding (<1%)
- Perforation (<1 %)
- Allergic reaction to sedation medications (<1%)
- Inflammation of the veins from the IV and medications
- Prolonged sedation (1%)

Colonoscopy with intravenous (IV) sedation may have some systemic side effects. Subjects will be monitored closely for side effects in the MCRU procedure room per University of Michigan Medical Procedures Unit guidelines. Subject's vital signs and oxygen saturation continue to be monitored (until they have recovered from conscious sedation). Subjects will be encouraged to call the phone number they are given on discharge from the MCRU for any concerns of side effects. The study team will also be notified of any side effects by the

endoscopist and/or from the record review. Subjects will be given discharge instructions (Appendix B).

6.2 Benefits

Subjects may not receive any direct benefits from participating in this study. Subjects will undergo a colonoscopy which may find large lesions despite being unprepped. An unprepped colonoscopy will not find small lesions and will not replace the need for a prepped colonoscopy. If abnormal or problematic areas are observed, the subject will be referred for follow up (to PCP or GI as appropriate). Any lesions or polyps found with be documented with endoscopic photograph and in the endoscopy report. No clinical biopsies will be taken. Subjects will be given a copy of their colonoscopy report no matter the findings.

7.0 STATISTICAL CONSIDERATIONS/SAMPLE SIZE/DATA ANALYSIS PLAN

This study is a prospective single center evaluation of microbiome composition and diversity, in the mucosa and stool of the right and left colon, as compared to spontaneously defecated stool. The microbiome will be quantified by the primary outcome of diversity. Other secondary outcomes would include differences in microbiome composition and diversity between right and left colon, colonic stool and colonic mucosal microbiome, spontaneously defecated stool, and FIT kit stool.

Primary statistical analysis will be accomplished by means of paired t-tests comparing mean outcomes between two sides of the colon or between mucosal and stool microbiomes on the same side. Past data on diversity shows the values to vary between 4 and 10. A rough estimate of standard deviation is range/4 = (10-4)/4 = 1.5. Based on this estimate, a sample size of 20 allows an effect size (mean difference on diversity) of 1 to be detected with 80% power and 5% level of significance. In this calculation, the correlation between the pair of measures (e.g. left and right sides) is taken to be 0.5, a moderate value.

8.0 STUDY MON ITORING

8.1 Adverse Event Definition

An adverse event (AE) is any untoward medical occurrence in a study participant. Adverse events will be reported to the IRBMed according to standard reporting guidelines.

8.2 Adverse Events (including serious (SAES) and non-serious)

AE Data Elements collected on case report forms

- AE reported date
- AE description
- Event onset date and event ended date
- Severity grade (serious or non-serious)
- Attribution to study agent (relatedness- not related, unlikely, possible, probable, definite.

- Expected or unexpected
- Whether or not the subject dropped due to the event
- Action taken with the study agent
- Outcome of the event
- Comments

Severity of AEs

AEs will be assessed according to the following table.

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

Activities of Daily Living (ADL)

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. **Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

8.3 Serious Adverse Events

DEFINITION: ICH Guideline E2A and Fed. Reg 62, Oct. 7, 1997 define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.

8.4 Data Security

Participants will be identified throughout the research record by a unique subject identification number (SID). Information which could identify participants, such as name, address, or medical record number will be stored in locked cabinets or stored electronically

in password-protected, encrypted files. Source documentation with participant identifiers will be stored in files separate from the files containing research data. Participant paper files will be stored in locked files at all times. Computer systems will be password-protected against intrusion; all network-based inter-site communications of confidential information are encrypted. An on-going computer-virus-protection program is available, used, maintained, and audited on all computers and pathways into the system, including good computing practice policies, screening of data files, executable software, diskettes, CDs, text macros, downloads, and other concerns as they arise.

8.5 Data and Safety Monitoring Plan

Each research staff member will undergo focused training on each task for which they are responsible, and will perform quality control for others similarly engaged. The study coordinator will produce monthly administrative reports that describe study progress including accrual, demographics, and subjects' status. Reports will also describe adherence to inclusion/exclusion criteria and study protocol, and any unanticipated problems involving risks to participants or others, and adverse events. Adverse events and unanticipated problems will be monitored continuously by the study coordinator and Principal Investigator, and reported to the Data Safety Monitoring Committee (DSMC) once per month. The PI and study coordinator will each be responsible for ensuring that unanticipated problems, including SAEs, are reported to the IRB in compliance with their requirements.

8.6 Cancer Prevention Data Safety Monitoring Committee (DSMC)

The Cancer Prevention DSMC reviews the progress of the study, including recruitment and retention of study participants, and side effects, including AEs and SAEs.

The DSMC is empowered with the authority to recommend a trial be suspended or terminated based upon concerns in any of the above areas of review. The DSMC reviews all serious adverse events and ensures that these events have been correctly reported to all institutional review boards, and that adverse events have been correctly classified as serious or not serious. The committee assesses the impact of these events upon the conduct of the clinical trial. Monitoring also considers factors external to the study, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study. Recommendations that emanate from monitoring activities are reviewed by the principal investigator and addressed. The charter for the DSMC is in Appendix C.

8.7 Cancer Center Data Safety Monitoring Board

This trial will be monitored in accordance with the NCI approved University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Plan and the Cancer Prevention.

At the regular Cancer Prevention DSMC meetings, the protocol specific Data and Safety Monitoring Report form will be completed. The report will be signed by the Principal Investigator or by one of the co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Board (DSMB) every six months for independent review.

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10.0 Appendix A: Stool Collection Kit Instructions

11.0 Appendix B: Procedure handout, procedure consent, discharge instructions

12.0 Appendix C: DSMC Charter

13.0 Appendix D: Case Report Form