Humans are superorganisms, made up of both human and microbial cells. Teeming inside and outside our bodies are trillions of microbes from thousands of different species of bacteria, fungi, parasites, and viruses. They can live on or within many different areas of the body, such as the skin, gut, mouth, eyes, and genitals.

In a healthy person, these microbes coexist peacefully, and some assist with essential physiological functions. For example, they produce vitamins humans lack the genes to make, help digest our food, regulate our immune system, and protect against other microbes that cause disease. But if something happens to disturb the balance of our microbial ecosystems—brought on by infectious illnesses, certain diets, or the prolonged use of antibiotics or other bacteria-destroying medications—our bodies may become more susceptible to disease.

In recent years, DCEG investigators led by Christian Abnet, Ph.D., Chief of the Metabolic Epidemiology Branch, and Rashmi Sinha, Ph.D., have developed a program to advance epidemiologic research on the microbiome, which refers to the collection of the gene sequences from the community of microbes in the human body.

“The microbiome is an underexplored aspect of human physiology,” said Dr. Abnet. “Recent progress in DNA sequencing and the Human Microbiome Project have laid the groundwork for powerful investigations. However, there are few well-conducted epidemiologic studies evaluating the role of the human microbiota and its related metabolites in cancer development.”

Drs. Abnet, Sinha, and colleagues have developed a multi-pronged approach to achieve this goal. They are evaluating new methods to develop best practices; exploring the feasibility of incorporating fecal and oral collections into new or existing cohorts; conducting etiologic studies; and evaluating associations of cancer risk factors (i.e. body mass index [BMI] and diet) with the microbiome and metabolome. In addition, Dr. Abnet, Anil Chaturvedi, Ph.D., and Neal Freedman, Ph.D., are leading a study to evaluate the impact of tobacco use on oral health and the oral microbiome.
Methods Development

With the advent of high-throughput methods for bacterial gene sequencing, DCEG investigators have taken a leadership role in adapting these methods to epidemiologic studies, standardizing them, and ensuring the approaches are sound.

“While microbiome research has grown exponentially over the past several years, findings have been difficult to reproduce,” said Dr. Sinha, an expert in the field of gut microbiomics. “The variability induced by sample collection and storage, DNA extraction, bioinformatic processing, and statistical analysis has not been systematically assessed.”

Tackling these issues head on, Drs. Sinha, Abnet, Emily Vogtmann, Ph.D., and colleagues have developed reliable methods of collecting fecal and oral samples that can be easily and cost-effectively implemented in cohorts. They considered several issues, such as preservation of a microbial signature in samples, stability of samples under field conditions, and preservation of samples to maximize flexible use in multi ‘omics’ assays.

In addition, Dr. Sinha says, “it is crucial to have appropriate standard reference materials for quality control of DNA extraction and sequencing samples in large epidemiologic studies.” She and collaborators have developed two types of standards, a chemostat produced in an artificial gut system, also called a “Robogut,” and artificial communities with known bacterial mixtures. Negative controls are equally critical and need to be included in studies.

The degree of standardization in microbiome measurement necessary for translation to large-scale studies is early in its development. Drs. Sinha, Abnet, and colleagues successfully initiated and completed a multidisciplinary collaborative project known as the Microbiome Quality Control (MBQC) Study that included numerous laboratories to investigate the impact of DNA extraction, PCR amplification, sequencing, and bioinformatics on microbial results. In 2014, they organized a workshop to present MBQC results to researchers from a wide array of disciplines ranging from molecular biology to statistics. In a 2017 publication, they reported a baseline investigation of variability from the MBQC project, in which blinded specimen sets from human stool, chemostats, and artificial microbial communities were sequenced by 15 laboratories and analyzed by nine bioinformatics laboratories. Investigators found that while each microbiome protocol step—including sample handling environment, DNA extraction, and bioinformatic processing—has the potential to introduce variation, they highlighted the potential effects of DNA extraction methods as a crucial target for standardization. “These results may guide researchers in experimental design choices for gut microbiome studies,” Dr. Sinha said. “We have set the stage for the next phase of addressing variability.”

Drs. Sinha and Vogtmann are also collaborating with biostatistician Jianxin Shi, Ph.D., to quantify the stability of the human microbiome over time. They are evaluating the impact of temporal variability in microbiome measurements on sample size requirements for etiologic studies. Results could have important implications for designing and analyzing future studies.
Incorporating Fecal and Oral Samples into Cohort Studies

DCEG investigators are exploring the feasibility of collecting fecal and oral specimens in a variety of existing cohorts and populations to develop the resources necessary to support large, prospective studies.

“Owing to a lack of cohorts with fecal samples, the majority of microbiome studies to date have used a case-control design,” Dr. Sinha said. “Although these studies have provided insight into the differences between the microbiome of those with and without cancer, they are unable to evaluate how the microbiome may be related to etiology.”

To bridge this gap, Dr. Sinha and collaborators have successfully collected fecal and oral samples in existing prospective studies in the United States and Bangladesh as feasibility studies.

Furthermore, in collaboration with the NCI Division of Cancer Control and Population Sciences, Drs. Sinha and Abnet organized the “Next Steps in Studying the Human Microbiome and Health in Prospective Studies Workshop” in 2017. The workshop was designed to encourage cohorts to collect fecal and oral samples and to enhance communications between multidisciplinary researchers. More than 200 international attendees discussed the best collection methods, inclusion of appropriate QC samples, various issues related to sequencing and bioinformatics, and the development of appropriate statistical analyses.

A person’s oral microbiota is composed of many unique bacteria. Participants in a study of variability in oral microbiota over the course of 10 months received this type of graph to see the unique bacteria in their own mouths.
Cancer Etiologic Studies

DCEG investigators have been pursing cancer etiology hypotheses tied to the human microbiome for several years. These investigations have included studies of *Helicobacter pylori* and gastric cancer, bacterial metabolites and colorectal cancer and breast cancer, and oral health and cancer.

Gastric cancer is currently the model of bacterially associated cancer; *Helicobacter pylori* has been classified as carcinogenic in humans by the International Agency for Research on Cancer. Research within DCEG has evaluated how risk factors for cancer, such as tobacco, body mass index, and pepsinogen levels, are associated with microbial diversity using samples collected in China. Dr. Abnet and colleagues found that among participants with esophageal squamous dysplasia, participants at greater risk for gastric cancer (low pepsinogen I/II ratio) had lower microbial richness than those at lower risk for gastric cancer. Associations were also detected with smoking history and body mass index in these samples. Ongoing studies are being conducted to further evaluate the impact of the oral microbiome on upper gastrointestinal cancer risk.

The association between the human gut microbiome and colorectal cancer (CRC) is another important focus of DCEG research. In a case-control study with data and fecal specimens previously collected in DCEG, investigators found that CRC cases had reduced fecal microbiome alpha diversity, increased carriage of *Fusobacterium* and *Porphyromonas* taxa, and reduced abundance of *Clostridia* taxa. Metabolomic analysis of the same specimens uncovered 41 small molecules that differed between cases and controls, providing insights on carcinogenesis.

A proposed association between the gut microbiota and breast cancer is supported by a small case-control study by former DCEG investigator James Goedert, M.D., and colleagues that suggested that postmenopausal women with breast cancer have lower gut microbial diversity. To further explore this relationship, Dr. Sinha, Vogtmann, and colleagues analyzed fecal samples from breast cancer cases, benign breast disease cases, and controls from three hospitals in Ghana. They found lower alpha diversity in both breast cancer and benign breast disease cases as compared to controls.

Poor oral health and periodontal pathogens have been associated with a number of cancers, mortality, and other chronic diseases, which suggests a role for the oral microbiome in the development of these conditions. Drs. Abnet, Sinha, Vogtmann, and colleagues are assessing the association between the oral microbiome and incident cancers of the bronchus/lung, colorectum, esophagus, head/neck, hepatobiliary tract, pancreas, small intestine and stomach, in nested case-cohort studies within the Agricultural Health Study, NIH-AARP, and the Prostate, Lung, Colon, and Ovary (PLCO) Cohort Study. This is the largest prospective evaluation of the oral microbiome and cancer incidence to date.

“This multi-cancer, multi-cohort project will allow us to test several hypotheses simultaneously, provide greater insight into the role of the oral microbiome in cancer at several sites, and allow us to evaluate common mechanisms across cancer sites,” Dr. Abnet said.
Microbiome Associations with BMI and Diet

There is increasing evidence that the gastrointestinal microbiome may play a role in relation to certain cancer risk factors, such as obesity and diet. Dr. Sinha and collaborators are investigating the association of the microbiome with BMI, processed meat, and drinking water nitrate intake.

In the Northern Finland Birth Cohort, individuals who have been followed since birth in 1966, the investigators found that fecal alpha diversity (i.e., microbial community richness) was lower in individuals with higher BMI at age 46. BMI was associated with 56 fecal metabolites. The genera that were most frequently correlated with metabolite abundances were Blautia, Oscillospira, Ruminococcus, Odoribacter, and Haemophilus.

In addition, Dr. Sinha and colleagues evaluated the microbiome in a dietary intervention study of processed meat and high-nitrate water. High meat nitrate/nitrite did not affect the microbial composition in the feces or saliva. However, high-nitrate water increased nitrate-reducing bacteria in saliva, including Capnocytophaga, Neisseria, and Kingella.

“Nitrate-reducing bacteria can convert nitrate into nitrite in the mouth, which when swallowed, can increase carcinogenic N-nitroso compounds in the gut,” Dr. Sinha said.

Tobacco Use and the Oral Microbiome

Tobacco use is known to be a primary cause of periodontal disease and may adversely affect oral health in other ways. Tobacco use is also known to alter the bacterial biota of the mouth.

DCEG researchers received funding from the U.S. Food and Drug Administration (FDA) Center for Tobacco Products through a trans-NIH–FDA collaboration coordinated by the NIH Tobacco Regulatory Science Program to study the impact of tobacco use on oral health and the oral microbiome. Drs. Abnet, Chaturvedi, and Freedman, Dr. Maura Gillison of MD Anderson Cancer Center, Bruce Dye, D.D.S., M.P.H., with the National Institute of Dental and Craniofacial Research, Dr. Martin Blaser of New York University, and Dr. Rob Knight of the University of California San Diego, are investigating how tobacco-induced changes in the bacteria of the mouth may lead to adverse health consequences, including some cancers. Using oral wash samples from approximately 11,000 participants in the National Health and Nutrition Examination Survey (NHANES) study, the team will investigate the association between types of tobacco use and the oral microbiome as well as the association between the oral microbiome and tobacco-related diseases. As part of this project, the investigators recently published an updated estimate of the percent of periodontal disease caused by tobacco.

“This study is the first to characterize the effects of tobacco on the oral microbiome in a nationally representative study population and allows for the examination of the association between the oral microbiome and tobacco-related diseases,” Dr. Abnet said.

“We will explore developing a biomarker of exposure,” he continued. “That means we’ll be trying to understand what the bacteria populations in the mouths of smokers look like compared to people who don’t use tobacco products. Our findings could be used to assess the effects of new and emerging tobacco products on the oral microbiome and human health.”
References


