KRESSER INSTITUTE FOR FUNCTIONAL AND EVOLUTIONARY MEDICINE

Gut: Microbiome Sequencing

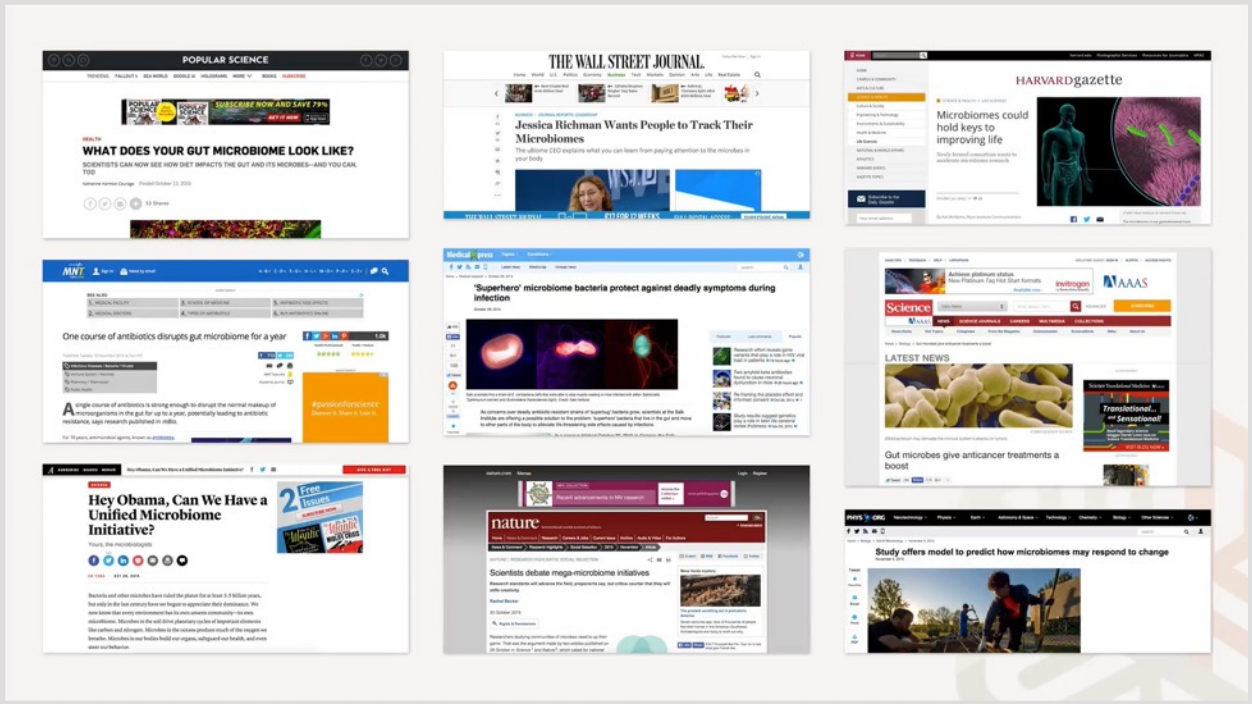
Hey, everybody, in this presentation we're going to talk about microbiome sequencing. In particular, we're going to talk about new tests that are offered through American Gut and uBiome, and others that are sure to follow, that sequence the microbiome. The tests focus on the gut microbiome, but they also offer sequencing for the skin microbiome, mouth microbiome and vaginal microbiome. They use 16S RNA technology to sequence the RNA of microorganisms. Robots load samples onto 96 little wells, they put chemicals into the wells, they heat them to break open the bacterial cells in the sample, and from this, a list of nucleotides and all 16S genes from all of the cells in the sample is produced.

This RNA technology allows for more accurate and comprehensive identification than previous stool-culture based techniques. Many species of bacteria in our gut are anaerobes and can't be effectively cultured, so this has revolutionized our understanding of the microbiome. However, because they only have samples of little stretches of the 16S genes, they don't know exactly which species are in you, just which lineages they are from. So to some degree, this limits the usefulness of this kind of testing in a clinical setting, which we'll talk more about shortly.

Before 2013, this kind of sequencing was only available in research settings, but it's now open to the general public via two organizations, American Gut, which now also has a UK arm called British Gut, and uBiome. American Gut and British Gut are research projects that have made testing available to the general public, whereas uBiome is a private company that provides microbiome sequencing as a paid service to consumers.

The big question here is what characterizes a healthy and sick gut microbiome, or for that matter, a skin or vaginal microbiome, and how does one move from the latter to the former? Again, we're talking here about the entire microbiome, because I'm going to focus on the gut because that's what most people are interested in when they use this test. So, the goal is to be able to compare large numbers of people who vary in different ways to find out which variables are significant and just how significant they are. For example, are the microbiomes of vegans, vegetarians, omnivores, people following a Paleo diet, different? How, and what's the significance of those differences? How does fermented fiber affect the microbiome? What about fermented foods? What changes do we see after taking antibiotics and other medications? What's the connection between different host microbiomes and diseases like Alzheimer's, cardiovascular disease, and multiple sclerosis?

These larger questions are what is known as first-order questions, but scientists are also interested in second-order questions like "does the effect of your diet depend on your ethnicity?" It looks like it does. Does the effect of having a dog depend on whether you live in a city or rural area? Looks like it does. Are certain species of bacteria beneficial for some populations but not for others? It looks like that's true. So, to answer these questions, we need a lot of data points, and this is why American Gut and British Gut, which are essentially a big research project, are open to the public.



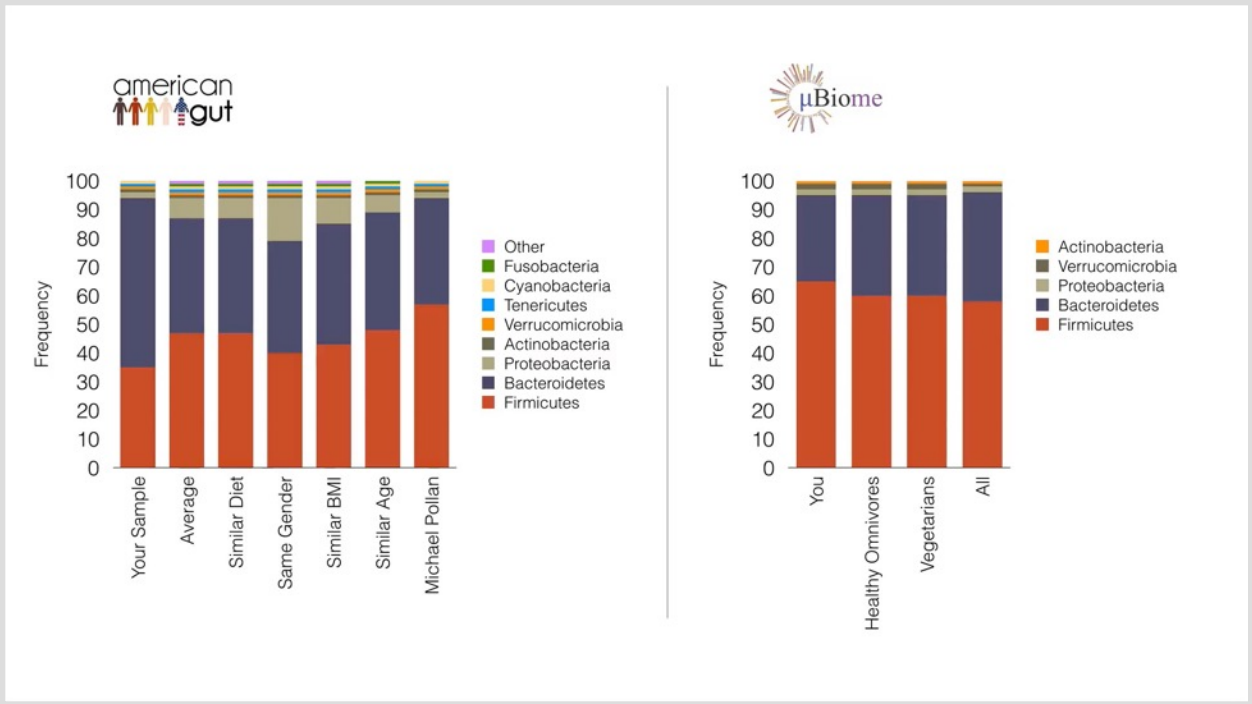
There's tons of enthusiasm about the ability to sequence the microbiome, as evidenced by hundreds of papers published each year in the scientific literature looking at the connection between microbiome and disease, and legions of media stories, you can see here on the slide, I've excerpted a selection of them. It's been a hot topic on Dr. Oz and other mainstream media outlets, but as Jeff Leach of American Gut put it—Jeff Leach is one of the directors of the American research project—we still know very little about the connection between disease and the particular culprits in the microbiome. The human gut is a vast and diverse ecosystem. There are over a hundred trillion organisms, which is a hundred times more stars than there are in our galaxy. As with any ecosystem, it's the community as a whole that's important, not any single member per se, and connecting the dots has proven to be a lot tougher than we'd hoped, and we still have tons to learn.

In addition, we still don't know what an optimal human microbiome even looks like. Most people living today do not have an original human microbiome because their lifestyle has changed so much, so it's difficult to say what is "normal" for humans at all anymore. Jeff Leach has been studying the Hadza hunter-gatherer population in Tanzania in an attempt to determine this because this is one of the last populations on earth that is still living relatively close to their original hunter-gatherer lifestyle, so Jeff is over there banking their stool and doing what he calls "poop archaeology" so that we can have at least some idea of what a normal human microbiome might look like.

But even if the Hadza have a microbiome that's more consistent with human norms over time, it doesn't mean that their microbiome is what's normal for people that are now living in an industrialized world. The microbiome is influenced by our environment, diet, exposure to pathogens, etc., so what's normal for the Hadza may not be normal or even beneficial for us. For

example, Jeff has found that the Hadza don't have much Bifidobacteria, which was a really big surprise because Bifidobacteria was universally considered to be one of the most protective species in people living in the industrialized world.

This leads us to another limitation, which is that there may not be such a thing as an optimal human microbiome. It's far more likely that what's optimal varies from population to population depending on numerous factors. Another big challenge when doing this kind of testing is how variable the results can be from lab to lab. In 2014, a science writer named Tina Saey sent split samples to American Gut and uBiome, so she took samples from the same stool that she passed and sent them to these two different organizations, and they were dramatically different, as you can see here on the slide:



On American Gut, Bacteroides, which is considered to be a beneficial species, were high, whereas Firmicutes, which are considered harmful, were low. But on uBiome, that was completely opposite. It turns out this isn't entirely unexpected, because American Gut and uBiome use different extraction techniques, and this along with variations in primers, collection techniques, and several other steps in the process has been shown in studies to strongly impact results.

So with all of this in mind, here are my recommendations for using this kind of testing. First, a single sample from an individual is almost meaningless according to Jeff Leach from American Gut. We just don't know enough about what a normal microbiome is for humans in general, or for a particular population or person. This kind of testing that's offered by American Gut and uBiome was really designed as a tool for comparing large numbers of people, not as a test for individuals in clinical settings that produce actionable results.

Having said that, there is one way that this kind of testing can be useful for individuals at this point, which is a time series. So, these are multiple samples that are collected over time to track the effects of interventions of the microbiome. So those interventions could be diet-related, they could be medications like antibiotics, or they could be supplements or lifestyle changes. Another way that these samples might be useful, and I put the stress on "might" there because we don't really know, is in detecting any particular extremes. So here we're talking about something like an overgrowth of a specific class of bacteria, the presence of something really rare of bacterial ratios that are significantly outside the distribution of the population to which the person belongs. But even this should probably be interpreted with caution, and I'm not even sure that I would treat on the basis of that data alone.

Finally, if you decide to recommend this testing to your patients, my suggestion is to use American Gut instead of uBiome. American Gut is a research project that's led by globally recognized leaders in bioinformatics, such as Dr. Rob Knight of UCSD. He's at the forefront of microbiome research, his lab and lab members continue to work on some of the most important microbiome studies around the world, their research appears regularly in leading scientific journals like ***Science***, ***Nature***, ***PNAS***, and ***PLOS ONE***. There are over 50 scientific collaborators on American Gut, including many of the key players in this field, like people who run the human microbiome project, the earth microbiome project, which are two of the largest initiatives attempting to characterize the human and global microbial environment.

American Gut makes all of their data open-source and publishes all of its protocols, and this is absolutely crucial in science, especially with a new frontier like this where many questions remain. uBiome, on the other hand, is a private company that does not disclose how they process the results, correct for microbial blooms, or analyze the data. I'm not suggesting that their methodology isn't good, but in a situation like this where there's already so much uncertainty, I think transparency and adherence to recognized standards and best practices are essential. Pricing is comparable: uBiome charges $89 for a single sample at the time of this recording; American Gut charges $99 for the sample. Both offer packages and time series options. I will say that one of the downsides of American Gut, I'm not sure if this is true for British Gut, but again at the time of this recording, the wait time for samples sent in to American Gut can be several months, whereas uBiome has a relatively quick turnaround. You can find out more about American Gut at AmericanGut.org, and you can also check out a podcast interview I did with Jeff Leach. It's in the additional resources section for this week's content. Okay, that's it for now, talk to you next time.