[MUSIC PLAYING]

ALISON MORRIS: I want to talk about microbiomes and clinical medicine. And I sort of view this like the Uber driverless cars, right? It's kind of science fiction. It may not be quite ready for prime time. Sometimes they crash. But it's happening, and we see them all the time. So we might as well know what's going on. And the reason I say this is happening now is it just takes a quick look at the internet to realize that the microbiome is all over the place.

So if you go and look, you see things like the microbiome diet, the scientifically proven way to restore your gut health, the microbiome cookbook, recipes to nourish your gut microbiomes. You can do things like the gut time lapse where you get three samples at a 10% discount to see what's in your microbiome over time. And then obviously supplements and probiotics are big business now. A quick look on Amazon, there are over 4,600 hits when you go and type in probiotics. Most of this has absolutely no scientific basis to it, but it's out there, and people are using it.

There's a ton of coverage in the lay press. These are some articles that have been in*The New York Times* over the past few years. There's been a lot of press in *The Wall Street Journal,* CNN, lots of other-- and the*Post Gazette* as well. And there are over 23,000 articles on PubMed. So this is really a rapidly expanding field.

So what I wanted to do today was give you a little bit of background on the microbiome, tell you some basic things about it so that you can look critically at some of these studies that come out, and then give you some clinical scenarios, one of which is actually happening now. And then the rest are things that may happen in the future that I see as probably being important and that you may see clinically in the next five to 10 years.

So just to start out with some definitions. The microbiota is a term you may here. And that's just the collection of microbial organisms that are in a defined environment, so in a particular site in the human body. It's important to remember that generally people will be referring to bacteria. And that's mostly what I'm going to be talking about today. But this really also includes fungi and viruses, which may play a critical role. The metagenome is just the genetic information of those microbes that are in the environment. And the microbiome is basically putting the microbiota and the metagenome together, so the bugs and their genetic content.

You'll really hear microbiota and microbiome used interchangeably. There is a difference because the microbiome is the genetic information as well. But it's not a distinction that you really need to worry about. So study of the microbiome is actually fairly old. It started back in the 1600s with Van Leewenhoek looking at his oral microbes under the microscope. But we have progressed dramatically since that time. As you all know, study of microbes has historically relied on culture-based methods.

But that's been really limiting that only really lets us get about 1% of the bacteria in a given environment because most of the bacteria cannot be cultured. So in the '90s with the development of DNA sequencing techniques that allowed you to amplify bacterial DNA without having to grow them, that was really when the field in the microbiome started to explode. And back in 2007, the NIH launched the Human Microbiome Project, which is really formed the foundation of this field.

This was a multi-center study. They studied about 240 adults, and sampled various body sites, and collected close to 5,000 specimens overall to start to say, well, what is in the human body in a normal state? And as I said, this has really formed a foundation and really dramatically changed how we think about microbes and the microbiome in the body. We now realize that it's actually another organ. It weighs about two kilos.

The majority of the bacteria are found in the gut, but we also have microbiome of the lung, the skin, the urogenital track. So it's throughout the body. Bacterial cells actually outnumber human cells. The exact number is debated. Some people say 10 to one, some people say less than that. But regardless, we're more microbe than human. And I think the key thing to really remember is that genetically, we're much more microbe so there are only about 22,000 human genes. As you know, they're fixed. You can't change your genes.

And they're pretty similar between people. So your genetics are about 99% similar to the person sitting next to you. In contrast, there are over three million microbial genes in the human body at any given time. And these are changeable. You can pick up different strains of bacteria. Your bacteria can mutate as they divide. And you only share about 10% microbial genetic similarity to other people. So this is a lot that has a lot more variability between people than the human genome.

The microbiome also has a lot of functions that we think are critical for health and disease. It's important in metabolism. Microbes produce vitamins and amino acids that we can't. The microbes in the gut produce short chain fatty acids, which are critical for health of the colon, as well as the immune system. Microbes are important for a host protection and immune development. They produce their own antimicrobial compounds, and they also in competition with other organisms can keep the bad bacteria at bay.

They're also very critical for development of your immune system. So dysregulated microbiome can lead to an overactive immune system. There's a big role for things like allergy, asthma, and auto-immunity. And there's a really interesting field developing in the gut brain access that seems to be bi-directional. The microbiome through production of these metabolic products through the immune function can influence the brain, things like autism, depression, other psychiatric disease.

But the brain and the nervous system can also influence the gut microbiome. So stress, or other things that can change transit time in the gut can change the bacteria that are there. So it becomes this really complicated relationship that we're just starting to figure out. So the microbiome has been postulated to play a role in almost any disease now that you can think of. Obviously, autoimmunity is a big thing. I'll show you some information about obesity, inflammatory bowel disease, skin disease, cardiovascular disease, and cancer, even things like flatulence, and halitosis, and mating preference, which may all be related. It's been shown to have a role of the microbiome.

There are a lot of influences of the environment on the microbiome. And we're starting to construct this theory of human health and disease in which the microbiome plays a role, biological systems like the immune system, and then the social and physical environment. So things like age, your diet, the environment you're in, smoking, genetics, pregnancy alters the microbiome. And the third trimester of pregnancy, the microbiome becomes more like with the microbiome you see in metabolic syndrome, which makes sense because that's what the body is doing.

Pets can change your microbiome. So the upper respiratory microbiome in dog owners is different than in people who own cats. And family members, so your microbiome looks more like the people that you live with than strangers. And then so this is really, I think all enters every phase of medicine. It's important in diagnostics for diagnosing disease more sensitively than we can with culture-based methods. Obviously, therapeutics are a big area. And there's a big impact on disease susceptibility and prevention.

The microbiome, as you'll see in a little bit, can influence the response to different therapies, including chemotherapy and other drugs. And it has an influence in the adverse effects that we see from different drugs. And it can also be used as a biomarker for different disease risks. There's a lot of promise for microbiome directed therapeutics. There's also a lot of hype right now. But there are many ways that the microbiome can be used therapeutically.

You all are familiar with probiotics, and there's obviously a lot of interest in that now. But there are other things like fecal transplant where you actually give fecal bacteria to recipients. Prebiotics can be used, so giving things that feed the bacteria, things like fiber, and that can alter the bacterial communities that are in the host. We can give people engineered bacteria. It's much easier to genetically alter bacteria than it is to do gene therapy in people. And so they can be genetically altered to do certain functions that are desirable and then given to people as probiotics.

We can also simulate the bacterial function, so things like the antibacterials, using their metabolic compounds that they produce, and things like that. Also very interesting is bacteriophages, which are viruses that infect bacteria. And these can be used to modify the bacterial communities and their functions. So I want to just take a minute to give you a little bit about how this is actually done.

I won't get too technical, but just to give you an idea, this is all based on amplifying this bacterial gene called the 26s ribosomal RNA gene. And this is present in all bacteria. So it allows us to amplify all the bacteria that are in any given population. And it has very highly conserved DNA sequences. So that allows the detection of all the bacteria. But in between those highly conserved sequences are very variable DNA sequences, so like a bacterial fingerprint.

So when you amplify this, it then gives you the ability to tell which bacteria are in a population. And when you do this, it looks like this. This is data from the Human Microbiome Project. And it just tells you basically the number and types of bacteria that are in a given body site or population. So these pie charts each color as a different type of bacteria, and their relative abundance is by how big the slice of the pie is.

And you can see that body sites look very different. There is no individual bacterial toxin that's found in every body site or in every individual. So it's pretty variable. Some other things you'll see, if you're looking at the literature on this, is a term called alpha diversity. And this is how we describe populations. It's based on which bacteria are in a sample. And this is in a this is a single person, OK?

It's based on richness and evenness. And richness is basically the number of bacteria. And evenness is how they're distributed. So you can see this is a very diverse population. It's rich. It has lots of different bacteria. And it's even because they're in roughly the same proportions. This is a very low alpha diversity population. You can see it doesn't have many bacteria, and it's dominated by one.

And you can think of this as a sort of c. diff is the classic example. And this is an example of some data. We have from patients in the intensive care unit. This is a lung sample. And you can see when they start out in intensive care, they have a nice mix of bacteria. And by day three, the lung has become dominated by staph aureus and this person went on to develop staph pneumonia. So a general loss of diversity is bad.

The other term you'll hear is beta diversity, and this is comparing differences in the bacterial communities and groups of samples, so either different body sites, or different clinical populations, obese, non-obese, things like that. And that just looks at the overlap in the kinds of numbers of bacteria in these two communities. And often it's plotted like this. This is a principal coordinates analysis. And this is, again, data from the Human Microbiome Project.

And so the closer a dot is-- each dot is a bacterial sample from a person. The closer together your dots are the more similar your bacterial communities are. So you can see all the orange dots are bacteria from the mouth in normal humans. And they're pretty far away from bacteria in the g.I. Tract. In contrast are the green and the red, or the skin and the nose. And those dots overlap much more. So it gives you a very quick visual way of seeing what bacteria are in a population.

So how does this relate to what you're seeing in your clinic? I think this is coming soon to the clinic. I get emails, not infrequently, people with various diseases saying is there something I can do for my microbiome? You'd be surprised, I've heard about a whole range of things that I never even thought were something that the microbiome was important. And oops.

So I want to start out with one that is actually happening. So you get a patient comes in and says, doctor, I can't get rid of my c. diff. What should I do? So you can tell them, eat a lot of yogurt, take lactobacillus, or get a fecal transplant. Now while yogurt and lactobacillus may not be harmful, a fecal transplant actually has data to show that it works. This is the general idea of a fecal transplant. You take stool from a normal healthy person and you transplant it into someone with c. diff or other issues.

There are a couple of ways to do. One, you can do it through a nasoduodenal tube. You can do colonoscopy or less commonly endoscopy. It's a pretty safe procedure. The risks are generally related to the procedure, so aspiration with the nasal tube, or the risks of a colonoscopy are obviously transferring infections, is the other big risk.

So there was-- the landmark study in this came out in *The England Journal* a couple of years ago. And they took 43 patients with recurrence c. diff, and they compared giving them a fecal transplant with donor feces to doing either oral vancomycin or vancomycin bowel lavage. They screen the donors very well for parasites or other infections, and they used nasoduodenal tube to transplant the stool.

And this is what they found. These are the percentages who had no relapse in the study period. Of those who got the fecal transplant, 81% didn't relapse. If they got more than one, over nine, there was over a 90% cure rate. And compare that to the vancomycin or the vancomycin bowel lavage, which had pretty low cure rate. So this is really a dramatic improvement over our standard therapies.

What was interesting, if you looked at that alpha diversity I told you about where the higher numbers of diversity are better, this is that alpha diversity in the donor stool. This is the alpha diversity in the patients with c. diff before they had infusions, so very low. And then this is their alpha diversity after, so increases to a normal level similar to the donors. UPMC actually has a fecal transplant program. This gives you a little bit of the history of it. It was started several years ago in the ID division, started by Scott Curry, and is now run by Tatiana Bogdanovic.

The first colonoscopic fecal transplant was performed in 2014. And the first nasoduodenal one was performed in 2015. We've since opened a volunteer stool bank. Before this, it was directed donors. These are the results of the first 19 patients that were done, a composite success rate of clearing diarrhea and clearing c. diff of close to 90%, so very similar to the studies I showed you and very successful.

This is being built up now. We're continuing with the directed donor program, but also trying to build this volunteer frozen stool bank in order to shorten the time to get a transplant, to allow transplantation of inpatients with c. diff, or patients coming from a distance. Also, Tatiana is working on doing a capsulated stool. So you'll just have to swallow pills, which has kind of less of an ick factor than the actual fecal stool transplants.

And this is Tatiana's a contact number if people have patients to refer, the easy-to-remember, FMT@UPMC.euc. You can send her an email, and there is a clinic open now. Other things that we're interested in doing with the fecal transplant program at UPMC, are increasing the indications both clinically and from a research standpoint. So should we be doing this in high-risk people with a first episode of c. diff, or for prevention and things like people who are getting transplants that we know are c. diff carriers?

We're looking at doing this for prevention and treatment of multi-drug resistant organisms, inflammatory bowel disease, oncology-- I'll talk about that in a minute-- metabolic diseases, and neuropsychiatric disorders. So I think these are things that all are going to be coming up in the future. In terms of the future of fecal transplant for c. diff overall, I think we're seeing an increasing popularity. There are multiple clinics throughout the country. But several questions remain. We don't know if there are particular donors or recipients that are better matches, like blood types.

We don't know if people should be banking their stool and get an auto transplant, when they get sick, if they should be getting stool from a relative versus a stranger. Are there particular species of the bacteria that are better, that we don't need to give the full stool transplant but just certain bacterial species? There's been one tria of this that just came out. This is through Seres Therapeutics. They looked at giving these bacterial spores that had been identified from healthy screen donors, and they had very promising phase one results.

But when they recently released their phase two results, their recurrence rates of c. diff were the same as placebo. So we don't quite understand which bacteria we should be giving yet. And it's important to remember that we're transplanting more than bacteria, right? If you're giving stool, you're giving bacteria, viruses, you're giving fungi, you're giving human cells from the colon. And so we don't quite understand what this is. And I think there are some other risks besides the ones I talked about.

I mentioned that the microbiomes involved in things like obesity, cardiovascular disease, psychiatric disease. And these are all things we could be transplanting and giving to people. And we don't really understand. There is no data on this yet, but there are some case reports of things like a woman who got a fecal transplant from an overweight donor rapidly gained 34 pounds in the month or year or so after a transplant. So we don't know if there may be these secondary effects. And as I go through these other cases, you may see that that can be a concern.

OK, so this is another potential clinical case that you can see in your clinic. This is not something that's happening now but I think has a lot of data to support that it may. So a patient comes in. Doctor, I just had a heart attack, but I really like steak. Can't you just give me something for my microbiome? And the answer is maybe. So gut bacteria are important for breaking down phosphatidylcholine, which is in red meat, and cheese, and eggs. And they break it down into this product called trimethylamine oxide, or TMAO. That leads to coronary vascular disease. So they're critical in this cycle.

And there's been a series of really elegant articles coming out of Stan Hazen's lab at the Cleveland Clinic, both looking at epidemiology, human studies, and animal studies. And this is some of the epidemiology data they have. They looked at over 4,000 participants. And the highest levels of TMAO in the blood were significantly associated with cardiovascular disease risk, so suggesting that the gut bacteria are important in these pathways, and also that this may be a biomarker for cardiovascular disease.

They then did this really cool study where they took healthy humans, and they gave them radio labeled eggs, OK? So they ate the eggs. And the normal at the baseline when they ate these eggs, they produced high levels of TMAO. They then gave them a month of antibiotics with Flagyl and Cipro and repeated the experiment, gave them another dose of eggs, their TMAO went away. So when they wiped out the gut bacteria, that could no longer produce TMAO. They then took them off the antibiotics, let their gut bacteria repopulate, gave them the egg challenge again, and the TMAO levels went back up to baseline, so really an interesting study of what the gut bacteria may be doing in cardiovascular disease with some interesting therapeutic implications.

So, of, course, everybody could just change their diet and not eat eggs and red meat. But that's harder to do. I think fecal transplant in this situation is not likely to be helpful given the risks. And the fact that diet will change your risk, change your microbiome back to what it was over time-- excuse me. My microbiome is somewhat altered today. I have a cold. So you could give people suppressive probiotics to dampen the bacteria in the gut, or there's-- thank you-- there's some studies now on this compound called DMB, which is an analog of choline that suppresses microbial TMAO production.

So this is under investigation and might be something that could be given to patients at risk for cardiovascular disease. Here's another scenario. Doctor, I think my gut microbiome is making me fat. Patient's asking for the garden salad, but his microbes are asking for the cheeseburger and fries. And this is something you may hear about because people are now able to get their gut microbes sequenced. And you get back this profile of how you compare to other people and how your microbiome looks compared to obese versus lean people.

So you could get someone coming in saying, look, I tested my microbiome. I look like I have an obese microbiome. What do I do? There's a lot of data-relating obesity in the microbiome. It's been proposed to play a key role. Diet rapidly alters the microbiome. So people who are vegetarians have a different gut microbiome than people who eat a lot of red meat. And this alters the efficiency of nutrient production. So if you have, say, a good microbiome and you eat a Big Mac, your bacteria will use more of those Big Mac calories and leave less of them for you. If you have a bad microbiome, your bacteria are not very metabolically efficient, and you get more of the calories of that big Mac. And so there are different profiles in the bacteria of obese and lean individuals.

And a lot of this data that we have comes from, again, a really interesting series of studies done from Wash U. One thing they did, they took four twin pairs discordant for obesity. So one of-- they were identical twins. One was obese, one was lean. They took fecal samples, and then they transplanted them into germ free mice. So these are mice that are reared without exposure to the environment. They have no germs of their own because they're bred in this germ-free facility. So they don't have any infections to start with.

And what they found was really interesting. So in the mice that got the fecal transplant from the obese twin compared to the mice that got the fecal transplant from the lean twin, there is a difference in weight gain. So even though they were on the same diet, same type of mice, the ones getting the obese stool got fatter, had more fat body mass. And then the ones that got the lean twin stayed lean.

And what was really interesting, if they then co-housed them. So they took the obese mice and the lean mice, put them in cages together, they saw that the obese mice got lean. And the reason that happened is mice are coprophagic. So they eat poop. So they do their own fecal transplant in the cage. And what was fascinating about it was that it seemed that the obese mice could become lean, but the lean mice were resistant to picking up the obese, the obese microbiome.

Another study that looked at this, the role of the microbiome in diet, and obesity, and in glucose response, was this large study that was done in Israel that came out last year. And it was a pretty complicated study, but basically what they did, they took 800 people. They looked at their genetics, their microbiome, did a lot of other anthropomorphic measures and clinical measures.

And they came up with this algorithm based on their genetics and their microbiome of what would be the best diet for them. And then they randomized them to either that diet or just a regular diet and found that they could have differences in the glycemic response and in weight gain based on a personalized diet resulting from information on their genetics and their microbiome. So it really says that we may be changing how we tell people to lose weight in the future.

So I think this raises questions about where are we going with this with treatment. Are we going to be able to treat obesity via the microbiome? We still need to know what are the key bacteria and the key functions and how they can be manipulated to treat obesity. Fecal transplant, again, has difficulties for a chronic illness. But probiotics, use of the metabolic products of the bacteria may be possible. Antibiotics targeting specific bacteria or tailoring the diets to the person's microbiome may help.

Just a note of caution, there has been a recent study that came out that said that the sample sizes that we're going to need to find an effect on obesity are going to be quite large. And so this may not be as useful as we're hoping, but I think it remains something that we're going to be seeing in the future. Finally, I just want to touch briefly on microbiome and chemotherapy as well as some diagnostics.

This is something that you may be seeing. The gut microbiome can identify cancer patients who are at risk for infections as they begin treatment, and also for other noninfectious complications. So things like colitis and pneumonitis can be influenced by the gut microbiome. The response to chemotherapy seems to be linked to the microbiome, at least for some, for some drugs.

So the immune checkpoint blockade drugs, the new anti-ctl4 and pd1 drugs that are used now and more in increasing numbers of malignancies, work by increasing the host response to the tumor. So they basically-- these checkpoint inhibitors basically take the brakes off of t-cells that can then go and attack the cancer cell. But the efficiency of that seems to really be changed by what the microbiome is. So the t-cells really, the immune system needs to have pre-exposure, and be revved up by exposure to the microbiome before getting these checkpoint inhibitors to be most effective.

So when they give checkpoint inhibitors in mice with melanoma, who either had been treated with antibiotics, or who are germ-free, they're completely ineffective, OK? So you need to have gut bacteria in order for these drugs to work. And certain microbiome characteristics seem to be better at triggering the immune system in the setting of these drugs to get an anti-tumor effect.

So some of the things that you may be seeing are doing fecal transplants prior to giving the checkpoint inhibitors, to increase their efficacy, or taking people who fail them, and giving them a transplant to see if you can get them to work. And the other thing I just want to mention is the utility of microbiome techniques for diagnosis, particularly of infections. So obviously, doing this kind of sequencing is much more sensitive than culturing techniques. And now it may be more rapid as well.

So there is, for example, a new sequencer coming out. And this is the sequencer. It fits in the palm of your hand. It's got the cute name of the MinION, or Minion. It comes from Nanopore. It's not quite ready for clinical use, but it's getting there. It gives very rapid, within hours, identification of bacteria that are in a given clinical sample, and it can give more detailed information about which pathogens are there, so resistance patterns, different strains, things like that.

And not only can we maybe use this for diagnosing infections, but maybe we can use this as an early warning of disbiosis in the microbial community, and then institute preventive measures. So if we see, we're losing diversity, and we're at risk for c. diff, we can maybe Institute therapy sooner, OK? And you could see something like this working for the number of people who come in with pneumonia who we never get an organism for. Maybe by doing this we could identify what the pathogen is and tailor antibiotics.

So I think there's a lot of potential for this in the future. And this technology, while not quite ready, is pretty close to coming into the clinic. So to summarize, microbial cells in DNA outnumber humans. They have a very complex impact on health and disease. Of the therapies we talked about, fecal transplant is currently approved and being used for recurrent c. diff. Death But I think the microbiome has the potential to impact many facets of disease, including diagnosis, progression, prevention, and therapy.

And I'd just like to mention, we do have a center that Dr. Star has mentioned that is over in the BST. And we're very interested in doing-- we're doing lots of research as well as clinical studies. And hopefully you'll be hearing more from this in the future. So I think the future of medicine is the microbiome is probably a bit of an overstatement. But I do think this is going to be a very important and emerging field in all facets of medicine in the upcoming years.