

UNC Charlotte researchers discover important links between gut bacteria, liver disease

Often, the more we learn about biology, the better we can treat disease — but the more complicated our understanding of disease itself becomes.

For example, you may know that you have trillions of bacteria living inside you, including the hundreds or thousands of species of friendly ones living peacefully in your gut, helping you digest food. But you may not know that the complex demographics — the ecology of it all, so to speak — of the rich and complex mob of organisms might be a major factor in whether or not you are the one-out-of-three people who might develop a major liver disease.

What? You don't know anything about your intestinal bacteria, not to mention how they affect your health? Don't feel bad — few if any people do. Some UNC Charlotte researchers are working hard to change all that, but (as people say on Facebook) — it's complicated.

INVISIBLE FRIENDS, ENEMIES

The lab of UNC bioinformatics

researcher Anthony Fodor recently announced a new finding that shows a strong relationship between complex microbial ecologies in human intestines and the common but serious medical condition known as fatty liver.

While this discovery may ultimately have a major impact on treating a common and important human disease, it also illustrates the paradox that recent research uncovering hidden biology of our bodies is actually making the goal of managing human health much more complicated.

First of all, though the discovery of a relationship between microbes in the intestines is puzzling news in itself, consider the fact that the bugs mysteriously connected to the liver ailment are themselves still pretty mysterious.

From studies of the human genome (“genomic” studies), we have learned that there is a mind-boggling diversity of benign bacteria in all of our intestines and that these populations can also vary almost infinitely from one human being to the next. Yet, this is very recent news. Most microbes, whether inside us or not, are surprisingly new to science, or are still completely unknown.


A little more than a decade ago, only a few thousand species of bacteria had been identified globally, though we now know that there may be that many species in a single human body. We were ignorant about bacteria because the only way to conclusively identify most bacteria and other simple-celled microbes was to look at their genes, and the only way to do that was to grow large, homogeneous colonies of them (to “culture” them) so that enough pure genetic material was available to test. And culturing bacteria

was a major stumbling block because most bacteria are amazingly fussy in what they eat and those dietary needs were — you guessed it — unknown.

Then along came “metagenomics,” a new field of genomic research that offered new tools to explore the vast and mysterious microbial world. Researchers like Fodor use metagenomics to look at genetic material taken from an environmental sample (rather than from an individual organism) and to detect the presence of different organisms within the sample, even though genomes of those organisms may be still unknown.

In particular, metagenomics uses a piece of genetic material (a gene known as 16S ribosomal RNA) that is known to be fundamentally similar in all cellular life but that also shows key differences between different species. By analyzing the variety of this one gene present in a sample, metagenomics researchers can tell how many different species (generally, this means bacteria) are present in a sample and, to some degree, what classification those species belong to.

The results are often surprising. An early test a decade ago found about three times more species in a small spoonful of dirt than were known to all of science at that time. Studies of the human body have found different populations living on the skin of the right elbow than on the skin of the left. Bacteria living in the human gut, which were once assumed to be a group of just a few species and similar from person to person, were found to make up large, complicated, interdependent ecosystems that are more distinct from person to person than fingerprints, even within the same family.



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MICROBES: UP TO SOMETHING

If the variety of bacteria inside us comes as a surprise, perhaps an even bigger surprise awaits. The question is, what exactly are they all doing in there?

We know that the kind of food we eat is important to our health and we know that having the right bacteria in our intestines is important in digesting our food properly, but we still do not know how our individual variations in gut bacteria might influence more specific health issues. In particular, we do not know how these bacteria influence how the substances we eat affect our organ systems.

In the condition known as fatty liver, fat deposits build up in the liver, with potentially serious health consequences for nearly a third of the American population. Fatty liver can be caused by alcohol abuse, obesity, hormonal changes and/or diabetes. Recent work has suggested that diet is also important in developing the condition, with strong indications that a deficiency in the essential nutrient choline might be partially involved in its occurrence. Choline deficiency also implicates genetics, since many people lack the genes to efficiently make choline internally.

Now, a new bioinformatics finding in Fodor’s lab has shown that the abundance or scarcity of certain types of bacteria in the gut may also help predict susceptibility to non-alcoholic fatty liver. The implication of the finding is that these groups of bacteria may be influencing the body’s ability to properly use the choline available in food, though the study does not examine the

specific metabolic activity of the bacteria involved.

In a metagenomic analysis of the microbial communities living in the intestinal tracts of 15 female patients participating in a study of the effects on liver condition from a choline-depleted diet, Fodor and his colleagues found a strong correlation between the relative abundance of two specific classes of bacteria and the development of fatty liver.

A report on the finding appears in the current issue of the journal *Gastroenterology*.

“Certain bacterial populations correlated very strongly with increased fat in the liver during a restricted choline diet,” said Melanie Spencer, a doctoral student studying with Fodor in UNC Charlotte’s new bioinformatics Ph.D. program and the lead author on the paper. “To us, it’s an amazing result because you just don’t see this clear a correlation in biological experiments in humans very often.”

The authors on the paper are Spencer, Fodor, Timothy Hamp and Robert Reid from the department of bioinformatics and genomics at UNC Charlotte, as well as Steven Zeisel and Leslie Fischer from the department of nutrition at UNC Chapel Hill.

ANALYZING GUT BACTERIA

Using metagenomics, the researchers analyzed the genomes of the patients’ gut bacteria before, during and after the patients were put on a choline-deficient diet.

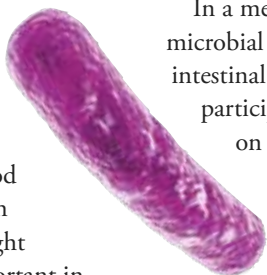
Because all patients consumed identical diets during the study, the researchers predicted that the initially distinct and complex communities of microbes in the patients’ intestinal tracts would react by becoming less distinct from each other. The researchers found instead that, though each of the patients’ bacterial communities did change a bit, each individual’s community still remained distinctive throughout the study.

“What we expected we might find would be that when we put the patients on exactly the same diets, everyone’s gut microbe mixture would begin to look similar, with the microbial communities converging. It did not happen — everybody was clearly individual throughout the entire study,” Spencer noted.

“So we also looked at how the patients’ microbes actually changed in pattern, even though they remained distinct from each other,” she said. “The patterns of change were very interesting. Some of the patterns were very distinct in themselves.”

The researchers noticed that among the numerous classes of bacteria present in each patient, variations in the populations of two particular groups seemed to correspond with variations among patients in the degree to which they developed a fatty liver during the period of dietary choline depletion. These disease-linked populations belonged to two special classes of bacteria — the Gammaproteobacteria and the Erysipelotrichi.

“Those patients with the highest abundance of Gammaproteobacteria at the beginning of the study seemed to have the lowest fatty liver development. The ones



with the least developed the most fatty liver,” Spencer observed. “Erysipelotrichi showed exactly the opposite association, though this relationship was not quite as strong. So there seemed to be change going on in opposite directions.”

In other words, when numbers of one bacterial group went up, the incidence of fatty liver went down — the bacteria seemed to be connected to controlling fatty liver. When numbers of the other bacterial group went up, the incidence of fatty liver increased — these bacteria seemed to be connected to the development of fatty liver

When the trends of Gammaproteobacteria abundance and Erysipelotrichi scarcity were combined and related to fatty liver development, the relationship became even stronger. The trends in the bacterial populations seemed to either strengthen or counter each other’s fatty liver—connected effect, thus increasing the likelihood that the bacteria were somehow related to the disease.

Finally, the researchers factored in individual genetic variations in the patients that affect internal production of the nutrient choline, expecting that should explain why some patients developed fatty liver and others did not. Surprisingly, the results showed that each person’s genetics did not entirely account for their fatty liver outcome.

When the researchers modified the analysis to include the abundances of the two bacterial groups and each individual’s genetics, the correlation between fatty liver development and these three factors

was nearly perfect. Further mathematical tests were performed to show that the correlations were not likely to be an artificial result of some bias hidden in the analysis.

“There was some concern that we were ‘over-fitting’ the model,” Spencer said, “so we tested it out and ran a million permutations, altering the bug abundance and subject association, to see if we could identify how many actually showed a higher correlation by chance. What we found is that the p values (measures for statistical significance) still held up. We can have a lot of confidence in the result.”

In other words, the statistical evidence for the connection between Gammaproteobacteria and Erysipelotrichi abundance or scarcity in the gut and the incidence of fatty liver were very strong. The bacteria seem to be having an influence that no one ever expected them to have on an organ far from where they live.



DEBATING THE NEXT STEP

The big question that remains for the team is why the two bacterial populations correlate so strongly to the development of fatty liver. Fodor sees a possible explanation, while warning against drawing specific conclusions without further study.

“We cannot yet assign cause and effect, but it implies that some bacteria are doing something that is making it easier for people to deal with a choline deficiency and for the liver to metabolize fat.”

Conversely, the bacteria whose high population levels correlate with disease may

be somehow removing available forms of choline from digested food. Fodor explains that further study will be needed to answer those questions.

“We’re debating what the next step is,” he said. “In some ways, this is a very specialized experiment because we are inducing fatty liver in a very specific way. In the general population, fatty liver is induced in many, many ways and not everyone who has fatty liver has low choline.

“It’s probably like Alzheimer’s or cancer, where there are many different causes for a disease that displays a common phenotype,” Fodor continued. “More research will be required to determine the extent to which bacterial populations play a role in fatty liver development in the general population, but our results strongly suggest that there may be a link in some people.”

Fodor and his lab continue to explore links between the unique characteristics of thriving, complex ecosystems we each have within us and the myriad of diseases that occasionally afflict us. Like the microbes themselves, the possible relationships are both still profoundly mysterious and bewildering in their potential complexity.

It’s a research effort that is just beginning, with enormous questions left to answer. That, of course, makes the scientists very happy — if not the rest of us who long for simple, clear explanations for everything that ails us. Science is getting there, but it’s going to be, well, complicated.



James Hathaway is research communication manager in the Division of Academic Affairs.

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