DESIGNER MICROBIOMES

TO WHAT EXTENT CAN YOUR MICROBIOME DEFINE YOU AND HOW CAN IT BE ALTERED TO OPTIMIZE YOUR HEALTH?

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Over recent years, the influx of news regarding scientific breakthroughs seem to suggest that the microbiome could be the answer to every health issue the world is facing. Despite this, the evidence and research has been trickling out slowly and there is still a lot we don't know about the bacteria we carry around with us every day.

The human microbiome field is renowned as one of the hardest and most researched fields, especially as research moves from academia to translational.

A few major projects have recently begun to publish their results, which appear to reinforce some of the hypotheses we have seen so far.

From the results of the American Gut Project to the microbiomes of athletes and surprising experimental results, we are going to look at the links between microbiome and health and the things we can do to alter microbiomes.

The results are in...

A team from the University of California San Diego, School of Medicine (CA, USA) has published the first results from the American Gut Project, the world's largest citizen science microbiome project. The results indicate factors such as diet, antibiotics and mental health can lead to variations in the bacterial composition.

"We observed a much greater microbial diversity than previous smaller studies found, and that suggests that if we look at more populations, we'll see more diversity, which is important for defining the boundaries of the human microbiome", explained Daniel McDonald, Scientific Director of the project.

It has been shown that citizen science projects can be successful in engaging the public in science. The open access nature of the work also provides a database for scientists to utilize to draw hypotheses, analyze and use to drive their own research.

The project was founded in November 2012, with the aim of gaining a better understanding of human microbiomes. As of mid-2017, 15,096 samples had been provided by 11,336 people from across 45 countries or territories including the USA, UK and Australia. To provide a sample, participants paid US\$99 and received a kit to provide a swab of oral, skin or fecal matter. *"It's really amazing that more than 10,000 people – members of the public who want to get involved in science whether or not they work in a lab or have a PhD – have mailed their poop to our lab so that we can find out what makes a difference in somebody's microbiome",* commented co-founder Rob Knight.

In order to identify the different bacterium present in a sample, the team utilized 16S rRNA, which is unique to bacteria. They also used metabolomics to assess non-living molecules and metagenomics for gene sequencing.

Trends have already emerged from the work, including links between the microbiome and diet, antibiotics and mental health. Those who consumed a wider variety of plant types had a more diverse microbiome, and those who were taking antibiotics, as expected, had reduced microbiome diversity.

Those with mental health disorders had more of their microbiome in common with those also with mental health disorders than their mentally healthy counterparts, with the research indicating those with depression may have particular types of bacteria that are more common than those without the disorder.

The project is still ongoing and the team is encouraging more people to get involved: *"The human microbiome is complex, but*

Figure 1. Population characteristics of the American Gut Project. (A) Participants across the world have sent in samples to American Gut, although the primary geographic regions of participation are in North America and the UK; the report that a participant receives is depicted. (B) The primary sample breakdown for subsequent analyses. Red denotes the reasons that samples were removed. (C) Between the two largest populations, the USA ($n = 6,634$) and the UK ($n = 2,071$), we observe a significant difference in alpha-diversity. (D) In a meta-analysis, the largely industrialized population that makes up American Gut exhibits significant differential abundances compared with nonindustrialized populations. Used with permission from [1].

the more samples we get, the sooner we will be able to unravel the many ways the microbiome is associated with various health and disease states", Knight said.

"The American Gut Project is dynamic, with samples arriving from around the world daily. The analysis presented in this paper represents a single snapshot, but we want eventually to go beyond making maps of the microbiome to making a microbiome GPS that tells you not just where you are on that map, but where you want to go and what to do in order to get there in terms of diet, lifestyle or medications".

This study has potentially solidified the link between the microbiome and health, and has also shown that a lot of environmental influences shape our microbiome.

However, before we can go about trying to improve our microbiome by changing our diets, medicine or lifestyle, it would be useful to find out how many of those factors actually do influence our gut bacteria composition.

If our microbiome composition is primarily established by genetic make-up, then efforts to alter and improve our microbiome could prove futile. However, if environmental factors

are the predominant influence, then we can begin making a difference now.

Nature or nurture?

Researchers at the Weizmann Institute of Science (Rehovot, Israel) have shown that a person's genetics only accounts for 2% of the variation between microbiome populations. The results of the study, published in *Nature*, suggest that other environmental factors such as diet and lifestyle are overwhelmingly the factors that contribute the most to gut microbiome diversity [2].

The microbiome has been linked with many common health disorders and diseases. Its composition is unique to each host and therefore linked to the health of each person. Changing the diversity of bacteria that reside in a person's gut has been shown to influence that person's health.

This study used a database of 1000 Israeli participants from a personalized nutrition program. Data collected included: genetics, gut composition, diet, medication and lifestyle. Israel

Figure 2. Genetic ancestry is not significantly associated with microbiome composition. Principal component analysis is shown in **A** and **B**. **(A)** Shows that there is an association between genetics and self-reported ancestry. **(B)** Shows no strong association for microbiome. **(C)** Shows that the distribution of phyla abundance of the microbiome is not associated with ancestry. **(D)** Shows boxplots of Bray Curtis dissimilarity for individuals with known grandparent ancestry showing no association between microbiome and ancestry. **(E)** Shows boxplots of Bray Curtis dissimilarity across shared ancestry fraction showing that microbiome similarity is not associated with ancestral similarity. Used with permission from [2].

was perceived as a perfect cohort for this type of genetic study due to the diverse genetic profiles of the country's population.

The researchers looked at the association between the different factors and composition of the participants' guts. For most of the factors, particularly the clinical parameters, there was more of an association between the genomes of the bacteria, not the human host. Therefore, changing the gut diversity could be therapeutic, as author Eran Segal explains:

"We cannot change our genes, but we now know that we can affect – and even reshape – the composition of the different kinds of bacteria we host in our bodies. So the findings of our research are quite hopeful; they suggest that our microbiome could be a powerful means for improving our health".

The group hope that their already extensive database can be added to in order to help answer more questions surrounding the microbiome and its effect on our health.

So, the environment is largely responsible for our gut microbiome composition. It's plausible that changing our diet and lifestyle could have a knock-on effect of altering our gut flora.

Now, where to begin? Let's start by looking at a group of people who are renowned for their healthy lifestyle and so, in theory, should also have a gut that's in good condition.

Could lifestyle be the answer?

A team of scientists from Harvard Medical School's Department of Genetics (MA, USA) has suggested that top athletes may benefit from particular bacteria in their microbiome, as well as tough training.

"When we first started thinking about this, I was asked whether we could use genomics to predict the next Michael Jordan", commented Jonathan Scheiman, a post-doc in the group. *"But my response was that a better question is: Can you extract Jordan's biology and give it to others to help make the next Michael Jordan?"* [3].

The team decided to look to the microbiome for answers. *"We are more bacteria than we are human",* continues Scheiman. *"The bugs in our gut affect our energy metabolism, making it easier to break down carbohydrates, protein and fiber. They are also involved in inflammation and neurological function. So perhaps the microbiome could be relevant for applications in endurance, recovery and maybe even mental toughness".*

Scheiman spent the week previous to the 2015 Boston marathon (MA, USA) and the week following driving around Boston, collecting fecal samples from 20 athletes and putting them on dry ice in his car in order to analyze how the microbiome changes during performance compared with recovery.

The group then sequenced the genomes of the bacteria present in the samples, finding an increase in the amount of a particular type of bacteria present in samples collected in the week following the marathon, in comparison with the week previous. These bacteria break down lactic acid, which is produced more during periods of high-intensity exercise.

Lactic acid can cause muscles to fatigue, leading the team to hypothesize that this type of bacteria could assist with fatigue prevention or faster recovery. They then isolated the specific bacteria from samples and commenced work to evaluate their effects on lactic acid levels and extent of fatigue.

The researchers also compared the microbiome of ultramarathon runners with rowers in training for the Olympics. From this, they found a type of bacteria present in the runners that wasn't present in the rowers, which breaks down carbohydrates and fiber.

Scheiman hopes the team can now develop natural probiotic supplements for athletes, with the launch of their own company Fitbiomics. *"I would like to think that a year after we launch, we could have a novel probiotic on the market,"* he concluded. *"But in parallel we'll also be expanding our cohort of elite athletes from numerous sports to generate a larger microbial data and*

strain bank of novel probiotic candidates. In essence, we're mining the biology of the most fit and healthy people in the world and then extracting that information to help them and others".

It's all well and good knowing that one person has good gut flora and that their diet and lifestyle probably contributes to that. But how can we take a good gut and put it into someone with a bad gut? One group has decided to look out to sea.

A new diet

Scientists at Stanford University School of Medicine (CA, USA) have manipulated the microbiome of mice by adding varying amounts of seaweed to their diet. The seaweed helps engraft the bacteria and the amount of it regulates the number of bacteria in the gut. The results of this study, published in *Nature*, further our understanding of the gut microbiome and may help to develop tools to fight disease [4].

The food we eat is often full of bacteria. When we ingest food, some of the bacteria remain in our system and become part of our microbiota; this is called engrafting. However, most of the time bacteria are simply flushed through the system.

It could be advantageous to engraft beneficial bacteria and grow them to help maintain a healthy gut and fight disease. However, the ecology of the gut is extremely complex and it has been difficult to establish colonies of bacteria until now.

Author Justin Sonnenburg explains: *"We're all endowed with a microbial community in our guts that assembled in a chaotic manner during our first few years of life. Although we continue to acquire new strains throughout life, this acquisition is a poorly orchestrated and not-well-understood process. This study suggests it could be possible to reshape our microbiome in a deliberate manner to enhance health and fight disease".*

To determine if introducing a new food would help engraft bacteria, the team first needed to find a bacterium that was rare enough to differentiate it from the millions already found in the gut. They chose a strain of bacteroides that was able to digest the Japanese seaweed Nori, and able to utilize a specific carbohydrate in Nori called porphyran.

Sonnenberg explained the choice: *"the genes that allow a bacterium to digest porphyran are exceedingly rare among humans that don't have seaweed as a common part of their diet. This allowed us to test whether we could circumvent the rules of complex ecosystems by creating a privileged niche that could favor a single microbe by allowing it to exist in the absence of competition from the 30 trillion other microbes in the gut".*

The team then had to try and introduce the new bacteria to the gut. They chose three mice for the experiment, two of which had their native microbiome replaced with that donated from two different humans. The third had a typical mouse microbiome.

When the mice were fed a normal diet the bacteria engrafted with mixed results. It was partially incorporated in two of the mice and rejected by one of the mice with a human microbiome. However, when the mice were fed with a porphyran-enriched diet the bacteria were successfully engrafted in all the mice.

The team also found that varying the concentration of the porphyran in the food varied the number of bacteria engrafted in the gut.

"The results of this dilution experiment blew us away. The direct effect of diet on the bacterial population was very clear", claimed Sonnenberg.

The group is very excited about what their discovery could mean for the future of microbiome research. *"Our growing ability to manipulate them (microbiomes) is going to change how precision health is practiced. A physician whose patient is about to begin immunotherapy for cancer may choose to also administer a bacterial strain known to activate the immune system, for example. Conversely, a patient with an autoimmune disease may benefit from a different set of microbiota that can dial down an overactive immune response. They are just a very powerful lever to modulate our biology in health and disease",* concluded Sonnenberg.

Adding bacteria to the gut is an obvious but challenging way to change the composition of the gut, but what about taking certain bacteria away? Luckily, modern medicine has developed antibiotics for this very job. What's more promising research has shown that this method could even help fight the biggest health challenge we face right now: cancer.

The big C

Scientists at the Center for Cancer Research at the National Cancer Institute (MD, USA) have revealed a connection between depleted levels of gut bacteria in mice and their NKT cell antitumor response in the liver. The discovery, published in *Science*, could prove crucial in understanding liver cancer and developing therapies to treat it [5].

Recently, there has been extensive research into the association between cancer and the gut microbiome. However, although the two have been linked, understanding the mechanisms behind the relationship has remained elusive until now.

The group started studying the basic association by treating mice with antibiotics, as author Tim Greten explains: *"What we found using different tumor models is that if you treat mice with antibiotics and thereby deplete certain bacteria, you can change the composition of immune cells of the liver, affecting tumor growth in the liver".*

They found that the mice treated with antibiotics exhibited fewer and smaller liver tumors and reduced metastases. Once this association had been established, the team began to look at the immune cells themselves.

The number of NKT cells had increased in the livers of the mice treated with antibiotics and further experiments demonstrated that these cells were responsible for the antitumor response. They also found that increased expression of CXCL16, was responsible for increasing the abundance of NKTs. But what caused the level of CXCL16 to increase? *"We asked ourselves, why do mice treated with antibiotics have more CXCL16 production in these endothelial cells?"* Greten said. *"That was the critical point, when we found that bile acids can control the expression of CXCL16. We then did further studies, and found that if we treat mice with bile acids, we can actually change the number of NKT cells in the liver, and thereby the number of tumors in the liver".*

The final piece of the puzzle was completed when the group found that a particular bacteria, *Clostridium scindens*, controls the metabolism of bile acid in the guts of mice.

Figure 3. Red algae from which Nori and porphyran are derived.

So the mechanism had become clear: antibiotics kill *Clostridium*, bile acid is metabolized less, CXCL16 expression increases, the number of NKTs increases and the NKTs fight the tumors.

This basic research has potentially far-reaching consequences for developing new treatments and therapies. It also indicates the importance of fundamental research to clinical advancement. If the microbiome can be successfully associated with the disease vs normal state, it will open up a lot of avenues for personalized medicine in the form of probiotics. Instead of vitamin supplements, people are now looking to probiotics, especially designer probiotics.

Whilst we are still a long way from a super seaweed antibiotic/probiotic smoothie that can turn you into Usain Bolt or cure cancer, these promising results do provide hope that established links are important for developing clinical treatment.

This knowledge could be utilized to get to a position where treating or altering the bacteria already inside us could be the best defence against pathogens and diseases that harm us.

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