Question & Answers during Webinar:

Prof. Haller:

Q: Can you please comment on the opportunities to treat *C. difficile* infections using fecal microbiota transfer (FMT) and the utility of secondary bile acids as biomarkers in the prediction of treatment success?

A: There is also one publication where *C. difficile* infection was efficiently treated by only using the supernatant of stool. That means there is a soluble environment which potentially can protect. This led to the identification of secondary bile acids which potentially inhibit *C. difficile*. This is followed up at the moment. I think we need to see if, in the end, we just need to use a certain liquid environment or if we stay at healthy stool donations for FMT. Transplanting from healthy donor works fantastically.

I wouldn´t fool around too much, at the moment. There are already first applications for biotherapeutic companies such as Sirius, for example, which come up with a small, well standardized consortium of bacteria to treat *C. difficile* infections and they failed in a phase II clinical trial. Although this is a good example, the world is not that simple.

Q: How do you analyze the different results you get from the V2 vs. V4 region?

A: That is a good question. If you use different regions of the 16S rDNA, you already make a selection of bacteria you want to analyze. This is like standing in a dark room and you switch on your torch, you have a spotlight. This is the selection of V-regions. At the moment it is not well comparable if people use V1/V2/V3/V4. In the best of all cases, and me and others work on that, you analyze the whole 16S gene. That eradicates the bias of certain V-regions towards particular bacterial taxa. You will get different answers if you analyze you samples via different V-region selection. There is no doubt.

Q: How meaningful do you think are transfers from human microbiota to mice? Which host factors can be ignored and which not?

A: There is a very recent review in Cell. If you do mouse studies you always need to know the limitations. A mouse is not a human. So you address mechanistic questions. The problem at the moment is that people make too large interpretations on certain model conditions. In our hands, the transfer from human to mouse means that you loose certain taxa, but the example I showed you is that for some diseases it really works that you can transfer a disease via transfer of microbial communities. Then you can mechanistically work with these model conditions. This is actually a publication we have in revision at the moment. It is an additional tool and when you know the tool well, you can learn a lot from it.

Q: You have addressed the need for standardization. How you are approaching the topic while technology is swiftly evolving. Do you see an agreement forming regarding the handling of fecal samples, especially one that is practicable in a clinical setting?

A: Unfortunately we are not there yet. There is a worldwide effort; European efforts, US efforts to form a consensus view on how to standardize. This starts with sample preparation: if you try to get the bacterial DNA it is a difference if you want to get gram-positive or gram-negative strains. In short,

there is a huge need to standardize, yet the international microbiome Consortium makes an effort to standardize, as well. Many different centers use different methodologies. I am involved now in a European consortium which tries to standardize these things and it will take a little while longer until the standards reach every applicant of the technology.

Q: What do you think about personalized nutrition based on the miocrobiome - knowing all this technical differences in the analysis?

A: Firstly, there are quite a few companies out there which are basically do single analysis of patients and normal people and they try to conclude something from it. This is a bad development as there is currently no way that I can make conclusions from a single analysis of a patient sample on what type of condition this person is or what type of risk this person carries. This is a trend I do not like as there are companies which misuse this. Second is, personalized means that you understand the interaction between something you give to the host and the response. Knowing we have very diverse microbial ecosystems it is very difficult at the moment to predict at a personal level. Yet, this should be the aim. Like in medicine, as well; if you look in cancer treatment, personalized medicine is the way to go. Maybe at some point we have better understanding of individual ecosystems and their function, then we can go into individualizing the situation. At the moment there is no way that we draw conclusions from a single patient or healthy individual derived dataset.

Q: How deep or strong is the association/causality between diabetes and microbiome from the current literature?

A: As I showed you: We see associations. In my opinion, the metabolic application of microbiome changes is still heavily debatable. There are potential implications, that the microbiome has an impact. I am still a bit skeptical. Some of our evidence shows a gap in understanding. I guess, that, at the moment, you cannot treat diabetes with fecal transplantation, for example. That would be a therapeutic application. Nevertheless, obesity changes the microbial environment. This association and causality needs to be stripped away from each other.