Intestinal microbiota assembly and dynamics in health and disease

Impact paragraph

In the last 10 years the human gut microbiota has drawn a lot of attention from the scientific community that through many research projects has discovered its role in human health such as in metabolism regulation, immune maturation, and protection against pathogen colonisation. Along with a deeper understanding of its physiological functions, scientists have linked changes in gut microbiota to many diseases. IBD, IBS, Celiac Disease, Type-1/-2 diabetes, atopic diseases, obesity and colorectal cancer are only few examples of a long list of diseases that together affect millions of people around the world and have all been associated to perturbations in the gut microbiota. Because of the vast societal impact of these diseases, the gut microbiota is the epicentre of a pharmaceutical "gold rush" worth 284.5M USD up to 2019. Beside its potential, the microbiome field is still very young and therefore tools specifically tailored for the unique challenges that its data analysis poses are only now becoming available. The shortage of analytical methods able to handle the compositionality, the sparseness and the high dimensionality of the data has resulted in the wide application of analysis techniques. These techniques are still inadequate for the intrinsic characteristics and dynamics of microbiome data. One example of this inadequacy is the current standard of using correlation indexes such as the Spearman's correlation. This coefficient is prone to spurious correlations if applied to compositional data. This issue, already addressed by Aitchison in 1982, remains essentially ignored. The use of inadequate analysis techniques is likely one of the reasons why the causal relationships as well as the mechanisms by which the gut microbiota affect human health remain largely unknown. In addition, many of the research papers published in this field to date are based upon cross-sectional study designs which can only partially explain the role of gut microbiota in health and disease.

The overall aim of this thesis was to provide more evidence on the benefit of the application of longitudinal designs and adequate analysis methods to elucidate the putative relationship between gut microbiota and health during early life as well as in children and adults.

In the first part of this thesis, we compared different methods to quantify the bacterial load in faecal samples as a way to deal with the compositionality of sequencing data. We however showed that the available quantification methods are either too laborious for high-throughput application in large population-based studies or too imprecise. These results are important as it shows the need to either develop other quantification methods or bioinformatic solutions to handle the compositionality of sequencing data. Better data analysis methods will therefore also have large societal impact on the results of microbiota studies and their role in health and disease.

In the second part of this thesis, we studied the impact of environmental and dietary factors, as well as stochastic factors, on the assembly and maturation of the gut microbiota in early life and how disruptions in these processes can contribute to the development of immune-mediated diseases. This time window in infancy is crucial, not only because it sets the conditions for microbial maturation, but also because the microbiota provides a stimulus for the adequate development of the gut and immune system. Our findings suggest that caesarean section delivery profoundly affects the colonisation pattern of the infant gut, mainly by limiting the transfer and expansion of maternal gut rather than vaginal microbiota. This might explain the lack of persistent effects of vag-

inal seeding, a procedure that is acquiring more and more popularity among mothers that deliver via caesarean section. Informing the general public and medical society on the detrimental impact of procedures such as caesarean section without a medical need might therefore have more impact than vaginal seeding.

Introducing a novel analytical method, joint modelling, to combine longitudinal microbiome data with survival analysis to study the time to disease onset, we demonstrated that alterations in the infant gut microbiota preceded the manifestation of atopic symptoms. Not only do these results add a new analysis technique to the toolbox of bioinformaticians to study longitudinal microbiome data, but they also provide insight into potential protective microorganisms in the prevention of atopic diseases. Faecalibacterium and Lachnobacterium were amongst the bacterial genera that were reduced among infants who subsequently developed allergic diseases and might therefore serve as candidates for potential next-generation probiotics to prevent these diseases. This is potentially very important for the health and wellbeing of future generations by creating an optimal starting condition of the gut microbiota leading to long term health effects.

In the third part of this thesis, we investigated the role of the gut microbiota in adults and its relation to the onset and course of two gastrointestinal diseases, Crohn's disease and Irritable Bowel Syndrome (IBS). Our findings show that while the microbiota of Crohn's disease patients differs from the microbiota of healthy individuals, the microbial community structure doesn't seem to play a role in disease exacerbations. We did however observe clear alterations in the gut microbiota of subjects who subsequently developed post-infectious IBS. To our knowledge this is the first time that gut microbiota alterations have been observed prior to the onset of active symptoms in IBS patients. Altogether, these findings substantially contribute to the causal role of the microbiota in the pathophysiology of IBS and provide further evidence that analysis of repeated samples over time can provide valuable knowledge to the field. The observed Bacteroides dominance in subjects susceptible to IBS development, moreover, provides strong leads for dietary intervention strategies in general to prevent functional bowel disorders.

To conclude, we demonstrate that robust analytical methods and adequate longitudinal study designs are essential to understand the role of microbiome alterations in health and disease and to subsequently develop strategies to modify or shape the gut microbiome to prevent or alleviate the disease burden caused by the global rise in non-communicable diseases.