Gilbert Greub[1], Claire Bertelli[2], Benoit Guery[3], Grégory Resch[4], Pascale Vonaesch[5]

The Human Microbiota Network of the Swiss National Centre of Competences in Research (NCCR) on microbiomes: objectives and main approaches

Studying the human microbiota is pivotal to improve our understanding of a number of infectious and non-infectious diseases. In Lausanne, microbiota related research started about 15 years ago when new generation technologies became available. However, it is only in 2018 that amplicon-based metagenomics was accredited in the diagnostic laboratory of the Institute of Microbiology at CHUV and was used for patients' care. Still, the indications remain limited and require investigations on the perimeter and limitations of this novel technology for targeted applications in the clinics.

To fill that gap, the critical mass of research on microbiota needed to be increased, which has been made possible with the new National Center of Competences in Research (NCCR) on microbiomes that started its activity on July 1st 2020. This NCCR co-directed by Jan van der Meer (UNIL) and Julia Vorholt (ETHZ) was initially composed of 18 different research groups, organized in 6 work packages (WPs), located in Lausanne (UNIL, CHUV and EPFL), Zurich (UNIZH and ETHZ) and Bern (UNIBE).

WP1, led by Gilbert Greub, is focusing on human microbiota, mainly in the gut, and currently exhibit nine research axis (see Table 1), i.e. four on specific diseases (D1 to D4) and five on method improvement or tool development (M1 to M5) to study the microbiota. The objectives of the six work packages is listed in Table 2.

Figure 1. Methods that are developed or improved by the NCCR human microbiota network.

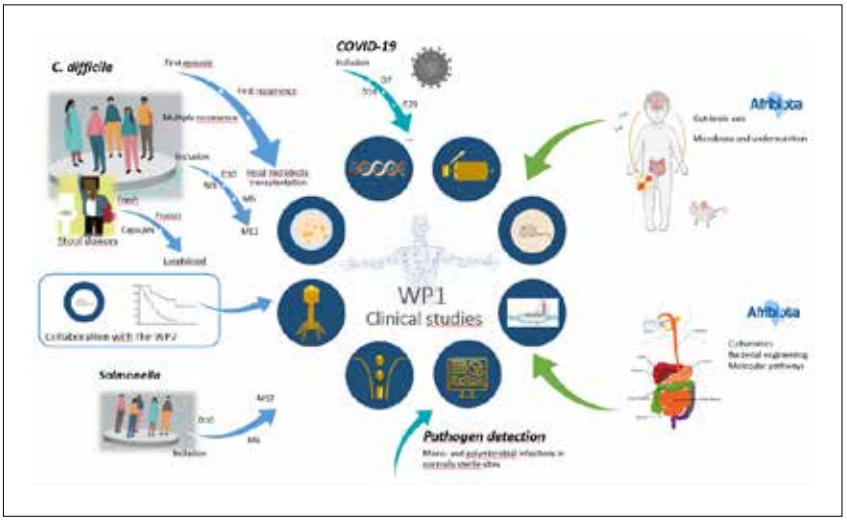

Figure 2. Main translational projects done by the NCCR human microbiota network.

Methods developed, implemented or improved by the human microbiota network of the NCCR

The methods developed in WP1 to study the human microbiota are summarized in Figure 1 and Table 1. Dedicated bioinformatics tools are being developed for diagnostic and research applications of metagenomics and other type of microbiota analyses. Fast (and relatively cheap) methods, such as flow cytometry, will be used

1 Prof. Gilbert Greub, Institute of Microbiology, University Hospital Center of Lausanne, Lausanne, Switzerland
2 Dr. Claire Bertelli, Head of bioinformatics, Genomics and Metagenomics Laboratory, University Hospital Center of Lausanne, Lausanne, Switzerland
3 Prof. Benoit Guery, Médecin chef, Service des maladies infectieuses, University Hospital Center of Lausanne, Lausanne, Switzerland
4 Dr. Grégory Resch, Head of bacteriophage research, University Hospital Center of Lausanne, Lausanne, Switzerland
5 Dr. Pascale Vonaesch, Assistant Professor, Department of Fundamental Microbiology, University of Lausanne

Project number[1]	Main objective	Possible deliverables
M1	Improve the metagenomics tools, including both wet lab protocols and bioinformatics pipelines; this project is initially focused on 16S rRNA amplicon-based bacterial profiling[2] but also aims to expand to shotgun metagenomics[3].	Provide efficient and robust diagnostic tools enabling the routine use of both amplicon-based[2] and direct metagenomics[3] in accredited laboratories
M2	Develop a culturomics[4] pipeline by combining automated inoculation, semi-automated colonies detection and MALDI-TOF identification. Define the robustness of culturomics as compared to amplicon-based microbiota profiling[2].	Provide a new modern culture-based approach to study the microbial composition, complementary to metagenomics and enabling isolation of key strains, that may be further studied.
M3	Implement phage production as a medical product for patient treatment. Develop phage-related tools to define the phages present in complex samples, such as the gut microbiota, and study the impact of phages on the diseases listed below (D1 to D4).	Improve knowledge and know-how on phages, phage production, and phage therapy.
M4	Implement flow cytometry wetlab protocols and bioinformatic pipelines to perform microbiota analyses and benchmark this new approach with PCR-based metagenomics. Identify flow cytometry signatures associated with gut dysbiosis.	Provide reliable and robust algorithm to Use flow cytometry as a routine tool in microbiological diagnostic arsenal.
M5	Develop new bioinformatics tools and adapt existing ones to improve the identification of virulence and antibiotic resistance encoding genes, as well as metabolic pathways.	Provide a comprehensive, robust and easy-to-use annotation tool for a flexible analysis both for research and diagnostic purposes with short time to results.
D1	Better understand the pathogenesis of Salmonella infection and whether the presence (n+1) or absence (n-1) of some gut bacteria may modify the natural course of the disease, including severity of the disease and duration of the bacterial shedding in absence of antibiotic treatment.	Define bacterial species associated with better or worse outcome and identify some protective bacteria that might be used as probiotics to improve patient care
D2	Better understand the pathogenesis of Clostridioides difficile and whether the presence (n+1) or absence (n-1) of some gut bacteria may modify the natural course of the disease, including severity of the disease, recurrent infections and treatment success.	Define bacterial species associated with better or worse outcome and identify some protective bacteria that might be used as probiotics to improve patient care
D3	Investigate the impact of SARS-CoV-2 infection on the gut microbiota and the impact of exposure to antibiotics and ICU stays on the antibiotic resistance profile of COVID-19 hospitalized patients	For subjects with severe SARS-CoV-2 infection, define strategies that might decrease the colonization with resistant pathogens.
D4	To investigate the pathophysiology underlying undernutrition and cognitive development in young children living in sub-Saharan Africa and describing the impact of the presence (n+1) and absence (n-1) of specific members of the microbiota on stunting/ cognitive delay	Define bacterial species and bacterial functions associated with better or worse outcome and identify potential protective bacteria that might be used as next generation probiotics to improve patient care

[1] D = diseases; M = methods; [2] «amplicon-based metagenomics» often use the V3-V4 region of the 16S rRNA-encoding gene and thus only allows «bacterial profiling»; [3] "shotgun metagenomics» and «direct metagenomics» are synonyms that both refer to «metagenomics sensu-stricto», i.e. providing sequences of all genes present in a given microbiota; [4] culturomics corresponds to high-throughput culture.

Worpackage	Thematics
WP1	Human microbiomes (translational microbiome research)
WP2	Microbiomes in animal systems
WP3	Plant microbiomes
WP4	Environmental microbiomes
WP5	Synthetic and engineered microbiomes
WP6	Data analysis and modelling

Table 2. The different work packages of the NCCR microbiomes, as outlined on the NCCR website (https://nccr-microbiomes.ch/research/overview/)

on different samples obtained through the various clinical projects and benchmarked to amplicon-based metagenomics metagenomics, which is already accredited and used in the diagnostic microbiology laboratory at CHUV. Conversely, more comprehensive analyses (metabolomics, shotgun metagenomics) will only be performed on a subset of samples selected from subjects of the main clinical cohorts.

Translational projects of the human microbiota network of the NCCR

As outlined on the NCCR website (https://nccr-microbiomes.ch/research/work-package-1/), two translational research projects are focusing respectively on Salmonella & Clostridioides difficile. These pathogens have been selected since they correspond to two very different clinical situations and both exhibit a yet poorly understood pathogenesis. However, we intend to further expand this «N+1 and N-1 approach» (see Table 3) applied to bacterial agents implicated in diarrheal diseases by also studying the impact of additional pathogens such as Campylobacter jejuni and Tropheryma whipplei on the gut microbiota of infected and colonized individuals. We also consider enlarging the current research by (i) better defining what is a normal gut microbiota, (ii)

Terms	Definitions
Microbiome	Literally, all genomes present in a given sample. Generally corresponds to all sequences obtained by direct sequencing of microbial DNA present in a sample, allowing not only a taxonomic profiling but also providing insight on virulence-related genes and genes encoding for various metabolic activities.
Microbiota	Diversity of microbes present in a given sample. Currently, the microbiota analysis is commonly done by sequencing the V3-V4 regions of the 16S rRNA encoding gene, directly providing information on the taxonomic profile of microbes, but not on their metabolic competencies or virulence-associated genes. Microbiota may also be performed using non-sequences based approaches such as microscopy (see dysbiosis below), flow cytometry, or culturomics.
N+1	Analytic approach where the effect of the presence of an additional microbe on the others microbes is investigated. Typically, the additional microbes may have a positive impact in an additive or synergistic way on the effect on a studied phenotype of the other microbes. Alternatively, this microbe may have a negative impact, which is than better studied by using the N-1 approach.
Healthy microbiota	Normal microbiota, without any pathogen and also without specific bacterial composition that might be detrimental. Please note that two commensals non-pathogenic bacteria could be detrimental and led to «dysbiosis» if upon their association they might, for example, complete a given metabolic pathway that could led to the production of a toxic compound. Antimicrobial compounds produced by some specific bacterial strains represents another example on how the presence of a single new strain may significantly impact the microbiota.
Dysbiosis	Situation where the microbial composition is detrimental to health. Definition of dysbiosis in directly related to the definition of healthy microbiota, since due to various causes the healthy microbiota may be modified and be dysbiotic. One of the best defined dysbiosis is the vagina microbiota, which is defined by the disappearance of the usual predominance of lactobacilli; lactobacilli are involved in the local acidification of the vaginal mucosa and are producers of antimicrobial product, preventing overgrowth of pathogens associated with vaginitis, vaginosis or other local dysbiotic condition.
Metagenomics	High throughput sequencing. This approach entered all microbiota research lab given the high quality of sequences obtained using approaches such as Illumina and the relative low cost of the instruments and the reagents.
Amplicon-based metagenomics	Relatively efficient and cheap approach to perform an analysis of the taxonomic composition of a given sample; based on a PCR amplification step, this approach is more sensitive than shotgun metagenomics; this approach generally use the V3-V4 region of the 16S rRNA-encoding gene and thus only allows «bacterial profiling» and does not provide any information on the presence of virus or on the presence of eucaryotes such as yeasts, filamentous fungi, protozoa or helminths in the sample.
Shotgun metagenomics	Analysis of all the genomes present in a given sample with a preliminary amplification step. Also called «direct metagenomics». The main limitations of this approach is the high cost and the low sensitivity (needs > 100'000 bacteria/ml)
Amplicon sequence variants	Sequences obtained by high throughput sequencing are compared and each unique sequence corresponds to an amplicon-sequence variant (ASV). Several ASVs may correspond to a defined taxonomic group (species, genus,family, order or class). ASVs ae used to perform bacterial profiling; however, to do a precise taxonomic profiling, the use of additional genes or at least the full 16S rRNA sequences is preferred. Such precision may be important since V3-V4 for example does not allow to discriminate the different streptococci species and Streptococcus gallolyticus was strongly associated with colon cancer – thus, such precise taxonomic affiliation may be of importance. The same is true with Lactobacillus, which includes some species associated with weight gain and others with weight loss.
Culturomics	High-throughput culture. This approach is now developed in Lausanne thank to the availability of both automated high-throughput culture system (BD-Kiestra) and MALDI-TOF (Bruker).

Table 3. Glossary on some microbiota-related concepts (non-exhaustive list)

studying more in depth the gut-brain axis and (iii) extending investigations towards non-infectious diseases, such as Crohn, ulcero-hemorrhagic rectocolitis, irritable bowel disease and gluten deficiency, as well as nutrition-associated diseases (undernutrition, obesity).

Conclusions.

The NCCR allowed hiring of two new Professors at UNIL (Claire Bertelli, Pascale Vonaesch) and one MER (Grégory Resch) in the human microbiota work package. Thus, initially composed of two group leaders (Gilbert Greub & Benoit Guery), the human microbiota network (i.e WP1 of the NCCR) includes now five group leaders who collectively sign this short article. Today, the five P.Is, all from UNIL, interact tightly together during the weekly work-in-progress meeting and several projects specific meetings. Noteworthy, the WP1 also interacts closely with several group leaders of other work packages (WP 2 to 6) on fruitful collaborations, and both (i) proposes the new tools developed by WP1 to others, but also (ii) beneficiates from tools and know-how of the other NCCR researchers.

Human microbiota research is still at its infancy and many clinical applications will likely emerge from current research performed within the NCCR with future benefit for patients' care.