

The Microbiome Studies in Metabolic Diseases have Advanced but are Poorly Standardized and Lack a Mechanistic Perspective

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During the last decades hundreds of studies have reported the association of Gut Microbiota (GM) with obesity and related metabolic disorders [1]. However, recently the microbiome studies were criticized about the lack of skepticism [2]. The author of the article questioned the role of GM in different diseases and asked whether the detected differences between the subjects biologically matter? We do believe that the role of microbiota in metabolic diseases is true. The impact of GM on human health should not be surprising since our gut shapes up the symbiotic habitat for at least 100 trillion microbial cells that count up to 100-fold more genes than our genome [1].

However, we consider that the current problem in microbiome studies is the lack of standardization, which disables the reproducibility of the results. In our experience, the different sample storage and DNA extraction methods cause variation in the amount of DNA and in the abundance of certain bacterial groups (Rintala et al., in preparation). In population studies genders and different age groups are being continuously mixed, which is incorrect since for instance the inflammatory mechanisms and association of GM with metabolic parameters vary between genders [3], and moreover, GM composition changes during aging [4]. Further, 16S sequencing yields thousands of OTUs, whose identification varies according to the database that is being used. This may create significant bias knowing the geographical differences in human GM [5]. Moreover, some of the sequencing results have not been reproduced using other methods, such as HITChip [6]. As it is required for other high-throughput analyses, i.e. Microarray, the 16S sequencing results should be validated using another conventional method, such as flow cytometry combined with FISH or with real-time quantitative PCR (qPCR). When qPCR was accepted as a standard method to quantify gene expression levels, The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines were established to promote consistency between laboratories. The same should be done to standardize the GM analyses.

Another question that one should ask is that how many associative studies do we still need to show that GM plays a role in different diseases? We believe that the era of associative studies on GM should now have reached its' end. The research must take one step forward and use the information gained from the existing studies to test the causative roles and underlying mechanisms of the microbes in different diseases.

An associative study can never identify the underlying mechanisms of a disease. Therefore the high-throughput sequencing and metagenome analyses should serve as a rationale to create animal models for validating the fundamental roles of the GM. If a specific bacterial species is associated with obesity, its' causal role in obesity should be confirmed by colonizing an animal with that species. A great effort in unraveling the underlying mechanisms that connect the microbes to certain diseases has been made by Drs Cani and Delzenne [7] and by the laboratory of Dr. Gewirtz [8]. However, setting up controlled animal experiments for such mechanistic studies lead to

another question: how much do animal experiments reflect reality? Most of the microbiome studies are made in germ-free or antibiotic-treated mice lacking the gut microbial community. These situations in human gut likely never exist, and therefore the observations obtained in microbe-free mice cannot be extended to humans. Other models, such as humanized mice should be considered for the experimental studies.

Finally, one important factor, which too often is ignored in GM studies, is the diet. The GM is readily influenced by diet [9], and thus their association with metabolic diseases may depend on the dietary composition. Many interventional human studies have failed to modify the GM composition in obese subjects possibly due to inter-individual variation of the initial composition before the dietary intervention [10]. Here, the metagenome analyses should identify the metabolic capacities of the enriched bacterial species in different diseases and conditions. This knowledge should be further applied to animal models and dietary interventions to elucidate whether and how specific dietary choices through modifying the abundance of GM can be used to treat specific diseases.

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