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Recent advances and remaining gaps in our knowledge of associations between gut microbiota and human health

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Abstract

The complex gut microbial flora harbored by individuals (microbiota) has long been proposed to contribute to intestinal health as well as disease. Pre- and probiotic products aimed at improving health by modifying microbiota composition have already become widely available and acceptance of these products appears to be on the rise. However, although required for the development of effective microbiota based interventions, our basic understanding of microbiota variation on a population level and its dynamics within individuals is still rudimentary. Powerful new parallel sequence technologies combined with other efficient molecular microbiota analysis methods now allow for comprehensive analysis of microbiota composition in large human populations. Recent findings in the field strongly suggest that microbiota contributes to the development of obesity, atopic diseases, inflammatory bowel diseases and intestinal cancers. Through the ongoing National Institutes of Health Roadmap 'Human Microbiome Project' and similar projects in other parts of the world, a large coordinated effort is currently underway to study how microbiota can impact human health. Translating findings from these studies into effective interventions that can improve health, possibly personalized based on an individuals existing microbiota, will be the task for the next decade(s).

Key words: Microbiota; Intestinal microbial flora; Probiotic; Gut; Intestine

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INTRODUCTION

In recent years, the commensal microbiota, including that of the small and large intestines, has received renewed research interest for potential associations with human health. The commensal microbiota consists of a diverse population of prokaryotic (eubacteria and archaea) as well as eukaryotic microbes that live synergistically within their human host. As early as the beginning of the 20th century, Metchnikoff proposed that putrefactive bacteria contribute to various disease processes and that modification of microbiota composition through consumption of viable microbes might help to improve health and longevity[1]. A variety of potential associations between gut microbiota composition/activities and health or disease have undergone scientific scrutiny. Evidence is mounting in support of an association between microbiota and diseases associated with failures in appropriate immune responses leading to excessive inflammation, such as atopic disease, inflammatory bowel disease and intestinal cancers[2,3,4]. During the next decade, findings from comprehensive microbiota studies currently underway can be expected to revolutionize the way we think about our microbial "friends and foes".

CONSUMPTION OF LIVE BACTERIA TO PROMOTE HEALTH

A wide variety of often milk-based fermented foods containing viable beneficial microbes, mostly lactic-acid bacteria and bifidobacteria, but also other bacteria

and fungi, have traditionally been part of the diet in many cultures. Various cultures share the belief that home-made products, such as yoghurt, curd, kefir, chal, kombucha *etc*, can help to maintain good health. Microbial cultures used for preparing these fermented products have sometimes been propagated for generations. They range from simple cultures that contain a few lactic-acid bacterial strains to complex consortia containing various bacteria and yeasts (kefir grains). More recently, commercial products claiming to contain beneficial bacteria that can establish residency in the gut (probiotics), fermentable substrates that enrich for beneficial bacteria (prebiotics), or mixtures of both (synbiotics), continue to expand their market share. Such products have been quite popular in Europe and Asia for a while but they are now also becoming more common in other parts of the world including the US. Although it is clear that there is significant potential for such products to help improve or maintain health, the research validating many of the current health claims is sparse. We discuss below some of the recent findings and future opportunities to advance this promising approach.

THE HUMAN/MICROBIOTA 'SUPERORGANISM'

It is well established that commensal microbial cells living in intimate contact with their human host far exceed the number of human cells. Bacteria belonging to a few phyla, particularly Firmicutes and Bacteroidetes, appear to dominate in most healthy individuals[5-7]. Estimates for the total number of bacterial species compromising the collective gut microbiome have recently been extended up to 40 000[8], but, due to the large amount of emerging sequence data, the bacterial species concept likely will soon undergo revision. Most gut microbiota research to date has focused on exploring the eubacterial community, but archaea (prokaryotes resembling bacteria but different in certain aspects of their chemical structure, such as the composition of their cell walls), viruses, fungi and other microbes can frequently be detected in intestinal contents[9-12].

The combined microbial gene pool, studied by metagenomic approaches, exceeds the complexity of the human genome, extending the metabolic abilities of the human/microbiota "supra- or superorganism"[3,13,14], which is the combined host/microbe consortium. Through its immense metabolic capabilities, the gut microbiota contributes to human physiology by transforming complex nutrients, such as dietary fiber or intestinal mucins that otherwise would be lost to the human host, into simple sugars, short-chain fatty acids and other nutrients that can be absorbed[5,15]. Furthermore, the microbiota produces some essential vitamins including vitamin K, vitamin B12 and folic acid, contributes to intestinal bile acid metabolism and recirculation, transforms potential carcinogens such as N-nitroso compounds (NOCs) and heterocyclic amines (HCAs) and activates bioactive compounds including phytoestrogens[16-18]. Differences in environmental factors, including diet, as well as hosts genetics are thought to contribute to microbiota diversity[18,19]. However, as genetically similar mice obtained from a dedicated breeding colony and fed the same amounts of the same defined diet develop striking differences in microbiota profiles[20], factors beyond our current comprehension or even random chance might contribute.

DISEASES ASSOCIATED WITH GUT MICROBIOTA DISTORTIONS

Distortions in any one of the microbiota functions or signaling pathways could potentially contribute to a wide range of diseases, including cardiovascular diseases (IBD) (bile-acid-associated regulation of serum cholesterol levels, chronic inflammation), diabetes (carbohydrate uptake and glycemic control), inflammatory diseases including atopic diseases, inflammatory bowel disease (inappropriate immune stimulation) and neoplastic diseases (carcinogen activation, chronic inflammation related hyperproliferation). Eloquent studies suggesting microbiota associations with obesity have recently received significant publicity[21-25] but other studies have refuted the existence of such an association[26]. Undoubtedly, the gut microbiota can contribute to differences in energy gain from fiber fermentation. The resulting small amounts of additional energy, if absorbed by the host, can over time, contribute to weight gain, and signaling from gut bacteria might contribute to fat storage. However, from a public health perspective, we might want to avoid shifting the focus away from a more direct path to avoid obesity: balance energy intake and output.

Changes in gut microbiota composition by probiotic supplementation of infant diets have been shown to reduce atopic disease[2,27]. Associations between the microbiota development in infants and health later in life have long been proposed[28,29]. Utilizing microarray technology to monitor microbiota, Palmer *et al*[30] recently reported changes in the microbiota composition in 14 infants during the first year of life, pointing to considerable temporal variation and distinct features in each infant.

IBD has been linked to microbiota composition in a variety of studies[3,7,31-36], and successful interventions using a pre- and/or probiotic approach have been reported. In addition to reports of differences in microbiota composition analyzed in fecal samples, the kinds and amounts of mucosa-adherent bacteria also seem to differ between cases with IBD and healthy controls[7,37-39].

Colorectal cancer (CRC) risk also has been proposed to be associated with microbiota composition through various mechanisms[4,40]. Pre- and or probiotics have reduced carcinogenesis in some but not all animal studies[41,42]. Dietary prevention of intestinal carcino-

genesis in APC^{Min} mice (mice that develop large numbers of intestinal tumors due to mutation in the adenomatous polyposis coli gene) was associated with correlated differences in overall microbiota profiles as well as with the presence of specific bacterial signatures[20]. Increases in the amounts of intraepithelial *Escherichia coli* (*E. coli*) in CRC patients have been suggested[43].

Interest has recently also been directed towards establishing a potential association between microbiota composition and both type 1 as well as type 2 diabetes mellitus. Brugman *et al*[44] showed that antibiotics affected type 1 diabetes mellitus incidence but, more importantly, that microbiota differed before the onset of disease in diabetes-prone rats that developed type 2 diabetes. Similar data have recently been reported in immune system-associated studies in non-obese diabetic (NOD)-mice[45]. Antibiotic-induced microbiota changes have also been shown to affect type 2 diabetes, but systemic effects likely contributed to this observation[46].

Current studies of associations between microbiota composition and disease suffer from a lack of understanding regarding the normal range of microbiota diversity on a population level. Furthermore, the presence of particular microbes or microbiota pattern has been studied almost exclusively in observational studies, in which differences in microbiota were evaluated between subjects suffering from the respective disease and normal controls. This study design does not allow us to distinguish if differences in microbiota composition are causing the disease or if they are simply a result of the changed gut environment in diseased subjects. Prospective studies evaluating microbiota composition in individuals before they develop disease will be required to attribute causality to potential associations between microbiota and disease. Because such microbiota studies would be expensive and time consuming, they should be designed as ancillary projects as part of larger cohort studies.

NEW OPPORTUNITIES TO STUDY GUT MICROBIOTA AND HEALTH

Powerful molecular microbiota analyses methods, including 16S rRNA sequencing through a massively parallel barcoded pyrosequencing approach, facilitate for the first time our ability to analyze microbiota in depth and in an efficient manner. Studies of gut microbiota interactions with metabolic phenotypes (so-called functional metagenomics) are now possible through the use of proton nuclear magnetic resonance (^{1}H NMR)-based profiling of fecal, urine or other extracts. Early results in this area that tried to correlated microbiota and probiotic supplementation-induced changes in its composition are promising[47,48].

Last year, the National Institutes of Health announced its roadmap Human Microbiome Project (HMP) with funding in excess of one hundred million US dollars, allocated to improve our understanding of associations between human health and microbiota at five major sites: nasal and oral cavities, gastrointestinal and urogenital tracts and skin[49] (http://nihroadmap.nih.gov/hmp/). Efforts to sequence the genomes of hundreds of human-associated microbes are currently underway and multiple projects that will explore potential associations with human health are currently being funded. European and Asian countries are undertaking similar endeavors and international efforts have been made to coordinate projects. It can be expected that the studies to determine the composition, activities and dynamics of the human microbiota and its overall genomic content, the human microbiome, will expand our ability to utilize microbiota for maintaining/improving health.

REMAINING GAPS AND CONCLUSIONS

Studies of microbiota composition have so far been limited to fairly small populations. We are clearly lacking an understanding of microbiota diversity on a population level and across various cultural and ethnic groups. Few studies have extensively investigated microbiota dynamics in adults; the causes for variations over time have not been well explored. The many interventions aimed at improving health parameters through microbiota modifications with pre-and probiotic supplements have often been short-term. Thus, effects of microbiota changes on long-term health are unknown. Furthermore, the types and concentration of pre- and probiotic supplements significantly vary from study to study, making firm conclusions difficult to draw.

To improve statistical power for defining disease-specific microbiota pattern, it is frequently necessary to combine results from various individuals into disease and control groups. However, it is crucial to recognize that inter-individual variations in microbiota composition may be so large and its statistical distribution so far from normal that combining individuals might not be appropriate. The true extent of microbiota variation will only be known after we have studied a sufficient number of individuals. Massive parallel sequencing technologies and the necessary bioinformatics tools to handle the resulting large datasets have been and continue to be adapted for human microbiota analysis[50,51].

To date, little effort has been made to standardize the microbiota analysis methodology used in human studies. Furthermore, the extent of the bias introduced by different sample collection, storage and analysis methods has only been superficially investigated. This makes it almost impossible to directly compare findings from different groups, limiting our ability to generalize findings.

Successfully correlating microbiota composition with disease risk, rather than correlating it with disease status only, will likely require large prospective epidemiological studies sufficiently powered to detect disease predicting microbiota differences, even with the predicted large inter- and intra-individual variation. Such findings could lead to future microbiota based preventions, which may have to be individualized based on the subjects' existing microbiota. It is also important to establish microbiota

changes that are caused by, but are not causally associated with, disease progression. Such knowledge might facilitate the development of efficient microbiota-based screening tests (IBD, CHC *etc*). We have all the reasons to be optimistic that, based on new findings, expected through the current large multi-national efforts to better understand microbiota, we will finally be able to 'domesticate' our own complex gut microbiota as a means for improving health.

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