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Microbiota: A Key for Healthy Aging

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A growing body of literature has suggested that changes in the composition of gut microbiota could be associated with age-related processes. Significant changes in the gastrointestinal microbiota in the elderly have been reported according to diet, drug use, and place of residence. Moreover, changes in microbiota composition in old age have been associated with immunosenescence and inflammatory processes that are pathophysiological mechanisms involved in frailty. A better understanding of the possible reasons for changes in gut microbiota in the elderly and the impact of these changes on health could be a basis for maintaining healthy gut function and healthy aging in the present situation with an increasing elderly population.

Key Words: Microbiota, Bifidobacterium, Aging, Frailty, Elderly

INTRODUCTION

Healthy aging is going to become an important issue, since the development of medicine has achieved unprecedented longevity leading to a skyrocketing proportion of the global population over 65 years of age. In Korea, the proportion of those older than 65 years is projected to increase from 13.1% in 2015 to 40.1% in 2060[1]. An increasing elderly population directly leads to a mounting socioeconomic burden because those within this age range have great susceptibility to age-related diseases and disabilities such as infection, musculoskeletal disease, malignancy, and degenerative brain diseases. For this reason, strategies need to be changed from treatment of disease to preventing or delaying age-related diseases and maintaining good health as long as possible to reduce the duration and severity of morbidity that precedes death.

Consumption of a nutritionally balanced diet and maintaining a healthy lifestyle have been the basis for healthy aging, and a growing body of evidence supports that the intestinal microbiota is closely linked to human health[2-4]. The gut provides the largest physical interface between the environment, including pathogen, and the host itself. Metagenomic and metabolomic methods are becoming more prevalent, making it possible to more comprehensively examine the composition and functionality of the intestinal microbiota related to diseases or aging[5].

In this review, we aim to briefly summarize the process of the establishment of the gut microbiota and the evidence of the impact of aging on the intestinal microbiota composition, with particular attention to potential common pathological conditions in the elderly. Subsequently we discuss the potential reasons for changes in the gastrointestinal (GI) microbiota profile, and review the probiotics focused on the age-related condition to find a clue for delaying age-dependent deterioration of the microbiota-host symbiotic relationship. The pathogenesis of specific disorders that could be associated with dysbiosis in the elderly will be included in the review to provide a hint of the potential applications and future perspectives of this field of research.

ESTABLISHMENT OF GUT MICROBIOTA

"Gut microbes are our life-long companions: they live inside us, the vast majority of them within our GI tract, in a complex and dynamic mutualistic relationship starting from our very first days of life."[6] During delivery, the infant gut is colonized by microbes of maternal, dietary, and environmental origin. The presence of microorganisms in the placenta or amniotic fluid[7] suggests a primary fetal colonization. Beginning in early infancy, interaction with these environmental microbes is pivotal for the development of the intestinal mucosa and the maturation of the human immune

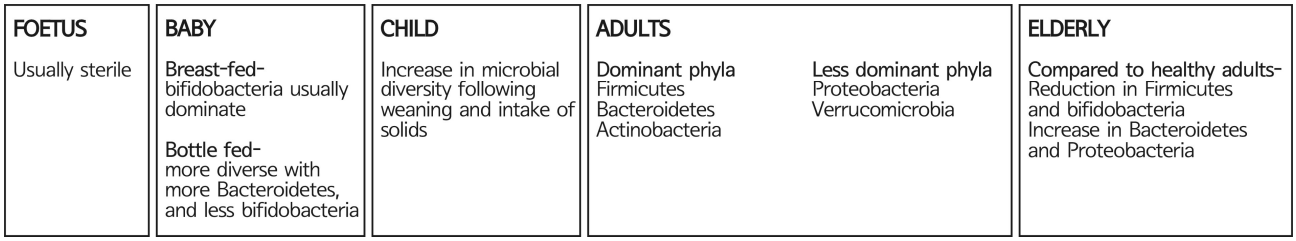

Fig. 1. Schematic summarizing changes in the composition of the gut microbiota through different life stages. Adapted from Duncan et al. Maturitas 2013;75:44-50[18].

system[8]. At weaning, the GI ecosystem is stabilized toward an adult-type phylogenetic architecture[9,10]. Actinobacteria, followed by Proteobacteria and Firmicutes are the main phyla in early childhood, characterized by low diversity and complexity[9,11]. The main changes in gut microbiota composition take place in the first stage of life, getting to a relative stability at 1–2 years old[12]. The adult-like structure of the gut microbiota is thought to occur after the third year of life[9,13] and reaches a total number of 10^{14}[14] microorganisms comprising bacteria, eukaryotes, viruses, and archaeal members[14,15]. At the phylum level, the gut microbiota is made up of 80%–90% Firmicutes and Bacteroidetes. At the species and strain taxonomic level, the diversity is very high in adults and is characterized by high inter-individual variability[14]. However, the functionality and metabolism of the gut microbiota is highly conserved[3]. The GI microbiota of a healthy adult has been regarded as relatively stable throughout adulthood, until aging and aging-related pathophysiological processes start to affect its homeostasis[16,17]. A schematic summary of the changes in the composition of the gut microbiota during a lifetime is illustrated in Fig. 1. Many of these commensals are essential to our health, helping to digest carbohydrates and create energy-rich short-chain fatty acids (SCFAs), synthesizing vitamins, and metabolizing toxins.

CHANGE OF GUT MICROBIOTA IN THE ELDERLY

Large interindividual variability in the microbiota, even in elderly subjects, and the reduced diversity of the intestinal microbiota in the elderly compared with younger individuals have been relatively uniformly reported[17,19].

Despite the downregulated microbial diversity, it has been accepted that Bacteroidetes and Firmicutes still constitute the dominant phyla in elderly, with lower contributions from the Actinobacteria and Proteobacteria phyla[17,20]. Nonetheless, the current knowledge of the age-related changes in the ratio of Firmicutes to Bacteroidetes is somewhat controversial, with results varying according to nationality and age of the enrolled subjects[17].

Regarding Firmicutes, members of the *Clostridium* cluster XIVa (a dominant group in the intestinal microbiota), which produces SCFAs, were found to decrease in elderly Japanese, Finnish, and Italian subjects, including centenarians[17,21-23], whereas an inverse trend was found in elderly German adults[23]. The species *Faecalibacterium prausnitzii*, which belongs to the *Clostridium* cluster IV and produces SCFAs, was significantly decreased in elderly Italians, including centenarians[16,23], although this result was not confirmed in other European populations[23,24]. A decrease in this important anti-inflammatory Firmicutes member of the gut microbiota has been relatively consistently reported in frail, hospitalized, antibiotic- and anti-inflammatory-treated elderly rather than in general elderly population[25-28].

Conversely, an age-related increase in Bacteroidetes was found in German, Austrian, Finnish, and Irish elderly[22-24,27], but this was not confirmed in elderly Italians, including centenarians[16,23]. Intriguingly, in the case of the Irish elderly, Bacteroidetes were found to be the dominant phylum instead of Firmicutes, which has always been regarded as the most abundant in healthy adults[24]. Currently, there is no consensus regarding the old-age specific gut microbiota profile due to the high interindividual variability, differences in diet and lifestyle, the unclear definition of the term "elderly," and different methodologies for stool sampling or analysis. However, some trends are repeatedly observed, such as the decrease of bifidobacteria, which is usually regarded as contributor to a health-favorable microbiome in the elderly population; this has been confirmed by several studies[17,29,30]. Unlike Firmicutes and Bacteroidetes, facultative anaerobes, including streptococci, staphylococci, enterococci, and enterobacteria[16,22,23,31,32], which are able to thrive in inflamed conditions and nurture the inflammation itself[33], are repeatedly shown to be increased in aged people.

Centenarians, extremely aged people, have microbiota still dominated by Bacteroidetes and Firmicutes (overall, 93% of total bacteria)[34], but the results remain somewhat controversial. In a European population, the microbiota composition of centenarians was similar to that of adults[16]. However, higher proportions of bifidobacteria were observed in centenarians than in younger elderly subjects from Guangxi in China[35]. Regarding species, *Bifidobacterium longum* was the most abundant in Italian centenarians followed by *Bifidobacterium adolescentis* and *Bifidobacterium bifidum*[36], but *Bifido-*

bacterium dentium was dominant in Chinese centenarians[37]. Different diets and regions could contribute to the different results.

POSSIBLE REASONS FOR THE CHANGE OF GUT MICROBIOTA IN THE ELDERLY

The mechanism of the change in the microbiota with age is not totally understood. Factors related to lifestyles of aged individuals including nutrition, mobility, and pathophysiologic change could lead to GI dysbiosis, which compromises the microbiota host mutualistic relationship.

Changes in taste[38] along with a reduced number of teeth and weakened chewing strength[39] strongly affects an elderly individual's diet[40], subsequently resulting in the intake of a limited range of nutrients to support microbial growth. Typically, the elderly decrease their intake of food containing fiber and proteins, such as vegetables and meat, and this is strongly correlated to changes in the phylogenetic and functional structure of the gut ecosystem[41,42]. The relationship between food and the composition of the gut microbiota has been recently demonstrated[43]. The microbiota of elderly people dwelling in residential care facilities was definitely different from that of the free-living elderly, even with the same ethnic and geographic conditions. The different dietary habits of the institutionalized elderly compared with the community-dwelling aged people can be associated with differences in the microbiota composition: the less diverse diet of long-stay residents resulted in a less diverse fecal microbiota profile. Both short-term and long-term dietary changes could affect the gut microbiota composition[44]. This can be a selection of specific microbial groups that are able to harvest as much energy as possible from the available substrates[34].

Intestinal motility may decrease with age[45]. This results in a longer transit time and alteration of the dynamics regarding nutrient turnover and consequently the microbiota profile.

Physiologic changes in the GI tract, as well as in other lifestyle components, may also influence the gut microbiota composition according to aging. Diminished physical activity compared with younger people also causes a reduced intestinal motility, which can lead to constipation, altered bacterial fermentation and metabolite production, and reduced bacterial excretion[17,46], possibly resulting in an excessive bacterial load challenge that is difficult to handle for an immunosenescent host[46,47].

Broad-spectrum antibiotic therapy or cocktail therapy with more than three antibiotics affects the entire GI microbiota[48,49]. The consequences of an altered GI microbiota after antibiotic therapy include increased carriage of antibiotic resistance genes, susceptibility to diarrheic syndromes, enteric infection, and altered inflammatory responses[50,51]. It has been reported that antibiotic therapy reduces overall bacterial diversity, affecting up to 33% of the microbial population and that it can also have an individualized effect on the GI microbiota[52]. More recently, a study showed the impact of antibiotic therapy prescribed during the previous month on microbiota composition of 185 older subjects staying in different settings, that is, long-term and short-term care, outpatient service, or community dwelling[53]. A significant decrease of *Bifidobacterium* spp. and a relative decrease of *Faecalibacterium* spp. was observed after use of nucleic acid synthesis inhibitors[53]. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) may influence the composition of the GI microbiota. A significant reduction in the Firmicutes, particularly in *Clostridium* cluster XIVa, and in the *Actinobacteria* was observed in older NSAID users compared with older nonusers and young adults[22]. Proton pump inhibitors, which are usually prescribed to elderly NSAIDs users could change the composition of the GI microbiota, leading to a less healthy gut microbiome[54,55].

THE IMPACT OF CHANGES IN THE GI MICROBIOTA ON HEALTH IN OLD AGE

The changes in the phylogenetic architecture of the gut microbiota that accompany the aging process could affect various aspects of human function, including a decline in metabolic and physiological functions, the defense capacity against pathogens, and even central nervous system (CNS) function (Fig. 2). This disruption of homeostasis leads to increased vulnerability to many diseases with aging.

1. Infection and Dysmotility in the Intestines

A markedly reduced bacterial diversity has been associated with active *Clostridium difficile*-associated diarrhea (CDAD), a major nosocomial complication for the elderly in hospitals and long-term facilities[56,57]. Since toxin-producing *C. difficile* species have also been detected in asymptomatic aged subjects, it has been assumed that a good commensal microbiota may play a protective role by preventing potentially pathogenic *C. difficile* from overcoming colonization resistance, proliferating, and producing toxins[57]. Within this context is the aim of fecal microbiota transplantation, a promising intervention for CDAD for restoring a protective healthy gut microbiota[58].

The production of SCFAs such as butyrate, acetate, and propionate by several members of the microbiota[59,60] is the essence of a healthy gut system.

Butyrate strongly stimulates the release of mucins that contribute to the physical separation between the microbiota and colonic cells[61,62]. SCFAs also enhance transepithelial resistance both *in vitro* and in an animal model[63]. Hippe et al.[64]

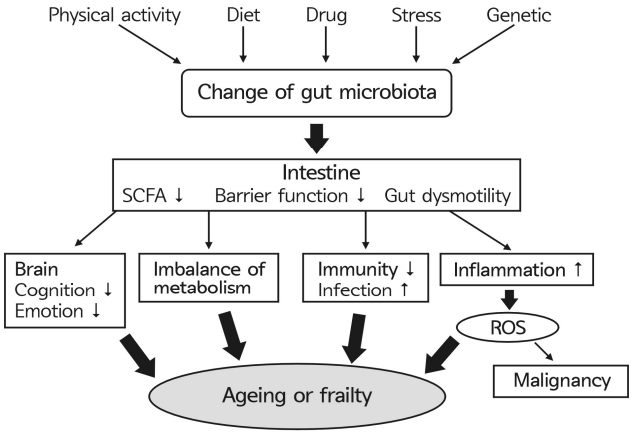

Fig. 2. Impact of changes in the gut microbiota on health in the elderly. SCFA, short-chain fatty acid; ROS, reactive oxygen species.

demonstrated that the elderly had significantly fewer copies of the butyryl-CoA:acetate-CoA transferase gene than younger adults. This functional decline was correlated with decreased amounts of *F. prausnitzii*, *Eubacterium hallii*, and bacteria belonging to the *Eubacterium rectale*/*Roseburia* group, which are all butyrate producers and belong to the bacterial groups found in lower amounts in centenarians[16]. This age-related microbiota depletion in SCFA producers may concur in compromising the integrity of the epithelial barrier. Such a weakened gut epithelium allows the passage of whole bacterial cells and their products and compromises immunological tolerance[65]. In particular, in the context of a weakened gut epithelium, the proliferation of Enterobacteriaceae and other gram-negative bacteria has been proposed to cause an excessive endotoxin challenge, leading to an abnormal inflammatory response[66].

The elderly consume less fiber than younger individuals owing to a declining sensation of taste and olfaction or redu ced income. This results in a reduction in butyrate formation and an increased formation of a variety of deleterious metabolites.

The decrease in fiber intake in the elderly may also trigger gut dysmotility. Methane formed by methanogens, which are more abundant in the elderly[15], can slow gut transit by influencing smooth muscle contractility[67], and conversely, high butyrate levels can result in an accelerate gut transit[68]. Slow bowel transit in the elderly will also affect the metabolic products in the colon and increase colonic pH compared with that in younger adults. Methane can contribute to the pathogenesis of constipation[69,70].

2. Inflammation and Aging

One of the recently recognized phenomena associated with aging is a persistent condition of systemic inflammation as part of the age-related decline of the immune system (immunosenescence) and of the pathophysiological process of senescence (inflamm-aging)[71,72].

It has even been suggested that chronic activation of the immune system might be directly related to the endogenous load of molecular signals expressed by the GI microbiota. A study investigated the association between the GI microbiota composition and inflammatory status in centenarians[16]. The increase of interleukin (IL)-6 and IL-8 levels in peripheral blood was linked to an enrichment in Proteobacteria and a decrease in the amount of some butyrate-producing bacteria, supporting the hypothesis that age-related modifications in the GI microbiota may contribute to inflamm-aging on the one hand or be affected by systemic inflammatory status on the other[16]. More recently, the same authors found that age-related evolution of the GI microbiota is characterized by a reduction in the genes involved both in the metabolism of carbohydrates and in SCFA production, whereas proteolytic functions are enhanced upon aging. These alterations promote the overgrowth of pathobionts that are able to sustain and to amplify the inflammation status in older subjects[73].

During the advancement of age, impairment of the gut-associated lymphoid tissue (GALT) and downregulated production of strain-specific secretory IgA, together with the reduced efficiency of the innate immune defenses, such as α-defensins, antimicrobial peptides, and mucus secretion, may result in failure to control the resident microbiota, allowing an uncontrolled microbial growth on the enterocyte surface. In this context, enterocytes could engage the activation of inflammatory cytokines and chemokines, forcing dendritic cells in the underlying GALT to drive the differentiation of effector TH1, TH2, and TH17 cells that induce a strong pro-inflammatory response[74]. Immunosenescence is also accompanied by a chronic, low-grade overall inflammatory condition (inflamm-aging)[71,75]. Inflammation favors the flourishing of pathobionts, a minor component of the healthy intestinal microbiota that in an inflamed GI ecosystem can overwhelm mutualistic symbionts and support inflammation[76]. A self-sustained pro-inflammatory loop that affects the entire microbial ecology of the GI tract created by pathobionts can contribute to the systemic inflammation and accelerate the process of inflamm-aging that is detrimental for host longevity.

3. Frailty

The downregulation of anti-inflammatory SCFA-producing bacteria could promote the inflamm-aging process in the intestine of the elderly. Moreover, the decline in butyrate-producing capacity may contribute to the development of degenerative diseases[77] and anorexia[78]. Indeed, an augmen-

ted ability to produce SCFAs as an additional source of energy for the host is predominant in the obese phenotype, both in mice and in humans[79,80]. Inversely, the less efficient SCFA production of the aged gut microbiota may contribute to the onset of malnutrition and sarcopenia in the elderly.

Frailty is a state of reduced physiological reserve and increased vulnerability to stress, which increases the risk of adverse outcomes such as falls, institutionalization, hospitalization, and death, due to a disorder of multiple interrelated physiological systems[81]. The correlation between the GI microbiota composition and frailty in older people has rarely been evaluated. van Tongeren et al.[28] compared the fecal microbiota composition of elderly volunteers with different grades of frailty, as assessed by the Groningen Frailty Indicator score. Fecal samples from volunteers with high frailty scores showed a significant reduction in *Lactobacillus* spp., *Bacteroides*/*Prevotella* spp., and *F. prausnitzii*. In contrast, the number of Enterobacteriaceae was significantly higher in the highly frail older subjects. More recently, Claesson et al.[43] found an association between the microbiota composition in the elderly and diet, inflammatory status, and clinical parameters. Moreover, they included a series of functional, cognitive, and nutritional measurements, most of which are part of the Comprehensive Geriatric Assessment[81], including the Barthel Motility Index, Functional Independence Measure, Mini-Mental Status Examination, Mini Nutritional Assessment, body mass index, and calf circumference. Loss of the community-associated microbiota correlated with increased impairment in the above-mentioned functional, cognitive, and nutritional parameters and supported a relationship between diet, the microbiota, and multidimensional health status. This indicated the diet-driven microbiota changes can have an impact on the decline in health with aging.

4. Metabolic Outcome and CNS

The microbiota in the GI tract is itself a source of metabolites and has an influence on the bioavailability of amino acids. The GI microbiota has been proposed to have an impact on adipose tissue and skeletal muscle energy metabolism, liver fat metabolism and hepatic steatosis, atherosclerosis and cardiovascular diseases, and enteroendocrine metabolism[82-84]. Of note is that SCFAs play a pivotal role in modulating the host energy metabolism. Given the tendency toward decreased production in SCFAs in the elderly, there would be a consequent imbalance in energy metabolism.

The microbiome-gut-brain axis, a 2-way communication that incorporates neuronal, hormonal, nutritional, and immunological signals between the CNS and the GI system[85], has been the basis for understanding the role of the GI microbiome in CNS diseases. Recent studies have provided evidence that the importance of the GI microbiome in a variety of CNS disorders including multiple sclerosis, Guillain-Barré syndrome, Parkinson disease, Alzheimer disease, and neuropsychiatric disorders (i.e., autism, depression, and anxiety)[86,87]. A significantly increased brain content of two phenolic acids produced by the GI microbiota after administration of grape seed polyphenol extract has been observed in a rat model. These phenolic acids potently hamper the assembly of β-amyloid peptides into neurotoxic β-amyloid. This suggests a potential role of the GI microbiota in protecting against neurodegenerative disorders[88].

5. GI Malignancy

A pro-inflammatory dysbiosis, together with decreased butyrate production in the intestine, has also been linked to an increased risk of colorectal cancer (CRC)[89-91]. Metabolic profiling studies using human fecal water extracts demonstrated a significant decrease of SCFA content in CRC[90,91]. The incidence of CRC increases in aged people, with approximately 50% of the western population developing colorectal polyps at the age of 70 and 5% of these polyps progressing to cancer[92]. The connection between CRC and aging raises the question of whether the age-related dysbiosis of the intestinal microbiota may be involved in CRC development. Colonic bacteria can affect the neoplastic process by inducing mucosal inflammation in the GI tract[89]. Then, the chronic inflammation can support carcinogenesis by inducing gene mutations that lead to the loss of antiapoptotic and anti-angiogenic properties. In particular, nuclear factor-κB is a key factor providing a mechanistic link between inflammation and CRC[93], and its activation by toll-like receptor ligands from intestinal microorganisms has been evaluated[94]. Therefore, sustaining inflammation in the GI tract by an aged intestinal microbiota may support CRC onset and progression.

Furthermore, the impairment of the barrier function of the aging gut microbiota could accelerate tumorigenesis by permitting persistent GI colonization by toxigenic bacterial strains. Certain bacterial toxins, such as toxin from toxigenic *Bacteroides fragilis* strains, colibactin, cytotoxin necrotizing factor 1, and cytolethal distending toxin from toxigenic *Escherichia coli* strains, can perturb the eukaryotic cellular signaling linked to cell cycle regulation and growth control, or directly damage DNA[6]. Besides toxins, lipopolysaccharides are also associated with metastatic colorectal tumor growth[95]. A better understanding of these pro-carcinogenic mechanisms may represent a crucial point in planning preventive and therapeutic strategies for CRC, which may include the modulation of the gut microbiota by probiotics, prebiotics, and/or antibiotics, with the aim of favoring bacterial species able to exert anti-carcinogenic activity.

Strong positive correlations were demonstrated between the quantification of each candidate by our quantitative polyme-

rase chain reaction assays and metagenomics approach (r= 0.801-0.934, all p<0.0001). *Fusobacterium nucleatum* was significantly more abundant in CRC than controls (p<0.0001)[96]. Resident microbes can induce inflammation, leading to cell proliferation and altered stem cell dynamics, which can lead to alterations in DNA integrity and immune regulation and promote carcinogenesis. Interestingly, a study in human patients and in rodent models of cancer have identified alterations in the microbiota of the stomach, esophagus, and colon that increase the risk for malignancy[97].

CONCLUSIONS AND FUTURE PERSPECTIVES

The bowel encompasses a diverse community where complex interactions between the body and the microbiota occur. There is a growing body of evidence that changes in the gut microbiota composition may be related to numerous age-related physiological processes.

Dietary habits, reduced physical activity, and the use of antibiotics, NSAIDs, and proton pump inhibitors can change the microbiota profile. However, there is no consensus about the definition of old-age specific gut microbiota profile due to the high interindividual variability, differences in diet and lifestyle, and the unclear definition of the term "elderly."

Nevertheless, it has been relatively consistently reported that there is reduced diversity in the microbiota in the elderly compared with younger contemporaries and reduced production in SCFAs. The ability of several members of the gut microbial community to produce SCFAs is an essential feature of a healthy gut ecosystem. Age-related microbiota depletion in SCFA producers may compromise the integrity of the epithelial barrier. Such a weakened gut epithelium allows the passage of whole bacterial cells and their products and disrupts immunological tolerance.

According to the literature, dysbiosis in the elderly could be associated with inflammation, frailty, GI malignancy, metabolic disease, and even CNS disease.

However, whether these changes in the gut microbiota profile are the cause or the effect of aging is still an open question. Therefore, more comprehensive evaluation including diet-microbiota-health interactions must be evaluated in the broader context of the genetic and lifestyle changes that accompany aging. Currently, prevailing internet of things (IoT) and big data collection technology may facilitate the evaluation of associations between microbiota composition and lifestyle or diet.

If a healthy microbiota composition is established, manipulation of the gut microbiota composition through the use of pro- or prebiotics and early detection of illness using regular screenings of individual microbiome profiles may be a strategy for healthy aging. This could be one of the approaches for personalized medicine.

Conflicts of Interest Disclosures: The researchers claim no conflicts of interest.

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