

ADDENDUM



## Identification of gut microbiome signatures associated with longevity provides a promising modulation target for healthy aging

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### ABSTRACT

The world population is aging, which poses a significant burden to the economy and health care system. As people age, so do their gut microbiomes. Age-related changes in gut microbiome have been reported, including decreased microbial diversity and increased Proteobacteria. Recently, we characterized the gut microbiome of a group of long-living ( $\geq 90$  years old) Chinese people. Interestingly, the diversity of their gut microbiome was greater than that of a young adult control group. We also identified several potentially beneficial bacteria enriched in the long-living Chinese group. These results were validated using data from an independent Italian cohort that included a group of long-living individuals. Other recent studies have found similar results. Here, we provide a summary of these discoveries and discuss their implications in healthy aging.

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gut microbiota; healthy aging; diversity; beneficial bacteria

## Introduction



The success of modern medicine, as well as better nutrition and medical care, has dramatically increased the human life span, leading to an increasing elderly population. By 2050, the global population of older people ( $> 65$  years old) is projected to reach 1.6 billion.<sup>1</sup> The aging global population poses substantial challenges to the economy, society, and the health care system.<sup>2</sup> However, longer lifespan (i.e., longevity) does not necessarily equate to healthy aging. As people age, they have a higher chance of developing a variety of diseases such as heart disease, stroke, hypertension, cognitive impairment, and cancer.

The gastrointestinal tract houses trillions of microbial cells that are known as the gut microbiota. The microbiota has coevolved with the human body and is critical to human health. It plays an important role in human health including, but not limited to, education of the host immune system, vitamin biosynthesis, protection against pathogens, and regulation of intestinal endocrine functions.<sup>3</sup> Changes in the gut

microbiome have been implicated in diseases such as inflammatory bowel disease (IBD), obesity, type 2 diabetes, and cancer.

## Gut microbiota in the elderly

Interactions between the gut microbiota and humans occur at each stage of life, beginning in utero and continuing through old age. The temporal shifts in microbial diversity and composition are critical to human development and health.<sup>3</sup> Many factors affect the diversity and stability of the human microbiome (e.g., diet, genetics, environment, and antibiotics). During aging, changes in physiology, diet, medication, and lifestyles lead to changes in the gut microbiome.<sup>4,5</sup> In general, gut microbiome diversity decreases during aging.<sup>3,6</sup> Regarding gut microbiome composition, however, contradictory results have been reported at the genus level, with some studies showing an increase in a specific genus in the elderly and other studies showing a decrease in that same genus.<sup>7</sup> At the phylum level, however, an increase in

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Proteobacteria has been consistently demonstrated across most studies.

### Centenarians as a model of healthy aging

The goal of healthy aging is to reduce and postpone age-related morbidities. Given the high morbidity rates among the elderly, it is difficult to find a cohort for healthy aging studies. Centenarians have consistently been used as a model for healthy aging studies because of their ability to delay, or even avoid, chronic diseases,<sup>8,9</sup> and their genetics has been extensively examined.<sup>10</sup> However, much was unknown about their gut microbiome until recently. The latest research discoveries have been summarized in several recent review papers.<sup>7,11,12</sup>

### Identification of gut microbiome signatures for healthy aging

In China, there are five regions known as “longevous counties,” and Dujiangyan, Sichuan is one of these regions. To study the correlation between the gut microbiome and healthy aging, we enrolled a cohort of long-living ( $\geq 90$  years old) people in Dujiangyan.<sup>13</sup>

We characterized and compared the gut microbiota in this long-living cohort with that of a younger adult group. Surprisingly, the long-living group had a greater gut microbiome diversity than the younger adult group, which is different from the conventional wisdom (i.e., that gut microbial diversity decreases during aging). Additionally, we found several potentially beneficial bacterial taxa enriched in the long-living group.

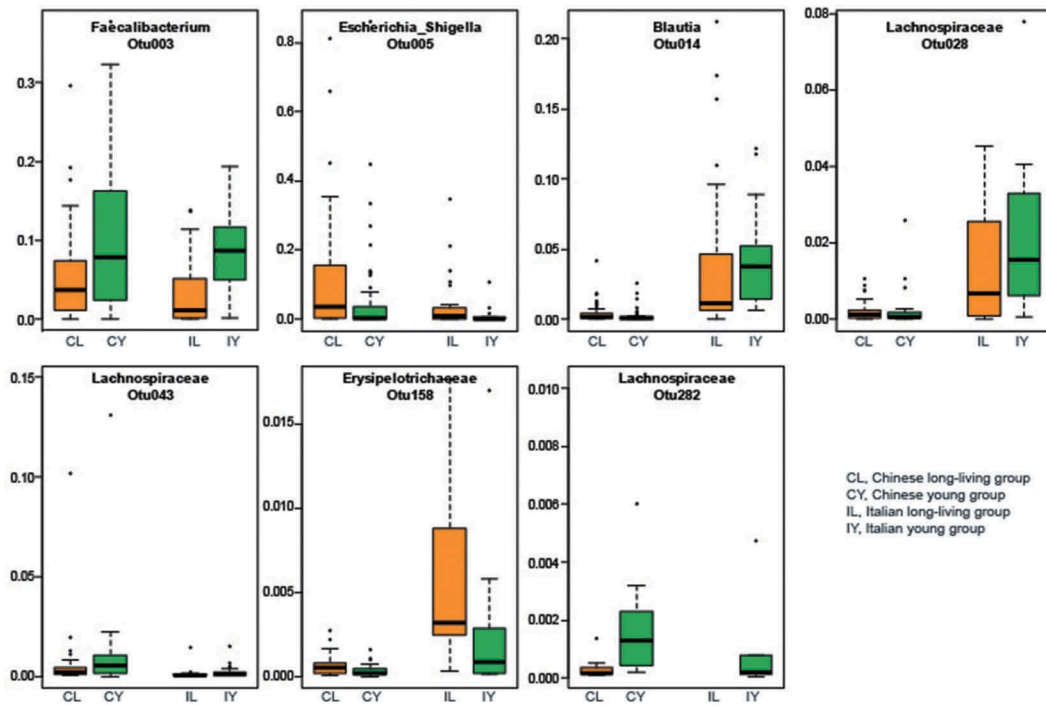
To validate these discoveries, we re-analyzed an independent Italian dataset.<sup>14</sup> Consistent with our results, greater gut microbiome diversity was observed in the long-living Italian group. The gut microbiome composition of the long-living Chinese cohort was significantly different from that of the long-living Italian cohort. This was very likely due to differences in diet, genetics, geography, and environment. However, among the top 50 most indicative features, 11 were shared between the two long-living cohorts. In our previous report, we showed six of the 11 shared features,<sup>13</sup> including greater alpha diversity

measures (e.g., Chao index and observed operational taxonomic units, OTUs) and an increase in potentially beneficial bacteria known as short-chain fatty acids producers (e.g. *Akkermansia*, *Clostridium* XIVa).

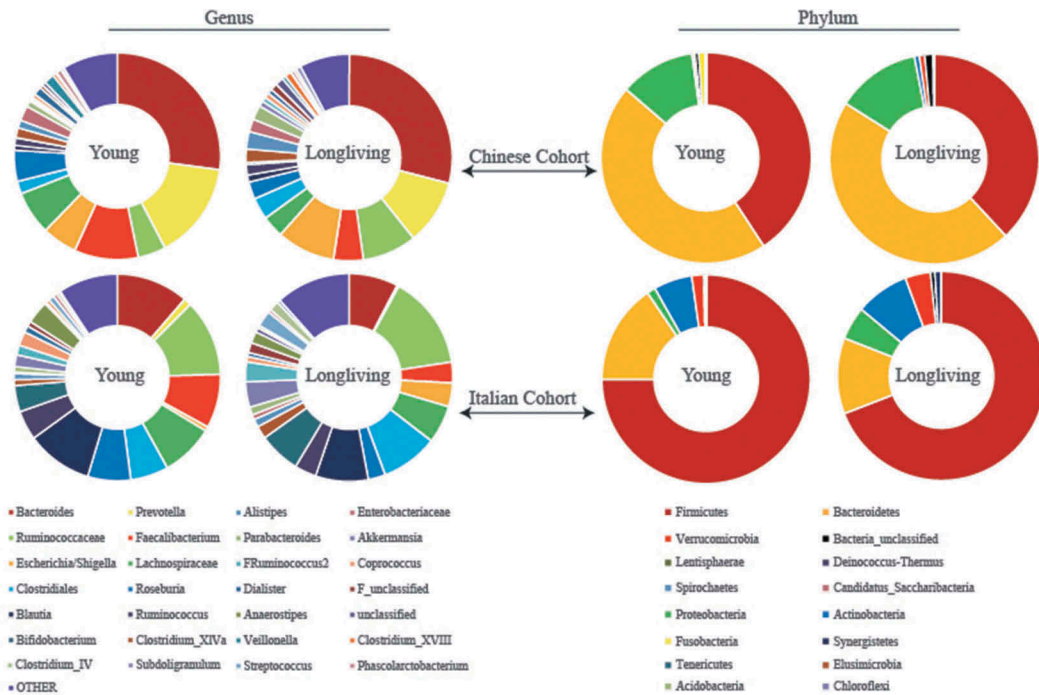
Figure 1 shows the relative abundance of the other five shared features. Of note, OTU5, which is associated with *Escherichia/Shigella* was increased in both the Chinese and the Italian long-living cohorts. Aggregated pie charts at the genus (Figure 2a) and phylum (Figure 2b) levels show the greater portion of *Escherichia/Shigella* and Proteobacteria, consistent with other recent reports showing increased Proteobacteria in the elderly. Therefore, increases in Proteobacteria during aging might be a common change in gut microbiome, regardless of typical elderly or long-living subjects. In addition, the relative abundance of *Faecalibacterium* in the long-living people decreased compared to the younger group in both the Chinese and Italian cohorts. These data suggest that although the discoveries in our previous study are promising, further studies are warranted to determine whether or not the gut microbiome plays critical roles in healthy aging.

### Gut microbiome diversity in the elderly

Microbial diversity has been used as a measure of a healthy microbiome.<sup>15</sup> Decreased microbial diversity has been correlated with increased frailty and diseases such as obesity, IBD, cancer, and type 2 diabetes.<sup>16–21</sup> It has been conventionally accepted that microbial diversity decreases during aging,<sup>3,6</sup> indicating an unbalanced gut microbiome in the elderly. Elderly people almost always have some gut-associated comorbidities.<sup>22</sup> Claesson, et al investigated correlations between gut microbiota, diet and health in the elderly. The microbial diversity of the long-stay group, elderly people in long-term residential care, was significantly lower than that of the community-dwelling group. The decreased diversity in the long-stay group was associated with increased inflammation (e.g., increased serum C-reactive protein), decreased healthy food diversity, and decreased overall health status (e.g., functional independence measure, FIM).<sup>5</sup> These results suggest it is important



**Figure 1.** Box plots of five shared features between the Italian and Chinese cohorts. We identified the top 50 features that differentiated the long-living and younger cohorts and in both our Chinese and in the independent Italian cohort. Among the 50 features, 11 were shared between the two cohorts. We previously showed 6 of these 11 features.<sup>13</sup> Here, we show the remaining five shared features.



**Figure 2.** Pie charts showing the average abundance of the bacterial taxa at the genus (a,) and phylum (b,) levels in the long-living and younger Chinese and Italian cohorts.

to stratify the elderly by their health status for studies focusing on the gut microbiome and aging.

The Chinese long-living subjects in our study were very independent and healthy at enrollment,

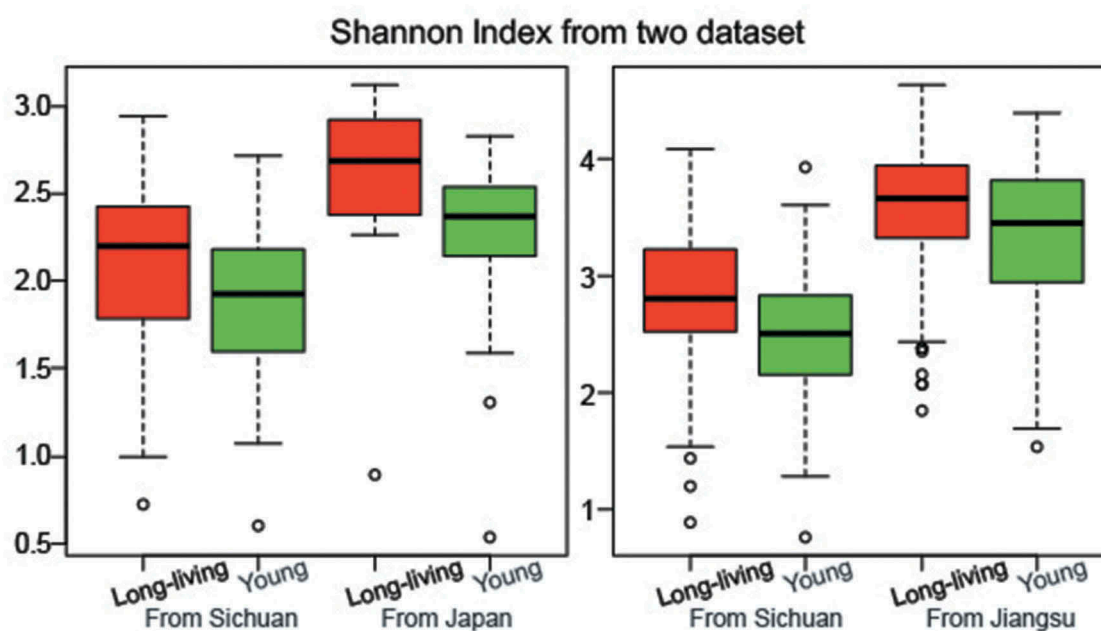
as demonstrated by their FIM values. FIM is a well-accepted metric to measure physical and cognitive disability. None of the people in this cohort were sick, hospitalized, or receiving any antibiotic treatments. These long-living people lived with family members, had a high quality of life, and enjoyed regular daily activities and games. Therefore, this cohort was not only long living but also a healthy aging cohort. The greater gut microbiome diversity observed in this healthy long-living cohort indicates a healthier gut microbiome in this group.

Two recent studies support our discoveries. Odamaki et al. performed a cross-sectional study that characterized 367 healthy Japanese subjects ranging from 0 to 104 years old.<sup>23</sup> Re-analysis of a subset of their data from subjects who had similar age range as the Chinese cohort in our study showed that the long-living Japanese cohort had greater gut microbiome diversity than the younger group (Figure 3a). In another recent study, Bian et al. examined the gut microbiome of a cross-sectional cohort of > 1,000 healthy Chinese subjects (mainly from Jiangsu Province, China) aged 3–100 years old and found that the gut microbiota composition of the healthy elderly group was similar to that of the younger people.<sup>24</sup> After re-analyzing their age-matched data, we found that the

gut microbiome of the long-living Chinese in the Jiangsu province was also more diverse than that of the younger controls, consistent with the data from our Sichuan cohort (Figure 3b). It is important to note that, although microbial diversity has been used as a parameter for a healthy microbiome, it must be interpreted in context.<sup>25</sup> For example, due to differences in sample collection, DNA extraction, and sequencing errors, diversity should be only compared between groups within a study; it is risky to compare the diversity between studies without considering the context.

## Conclusions

Our study shows that the gut microbiome of Chinese long-living people is more diverse than that of a younger group. This finding is supported by two additional recent studies.<sup>23,24</sup> We observed enrichment of several potentially beneficial bacterial taxa in the long-living cohort, members of which are short-chain fatty acids producers. However, we also noticed the decrease of certain OTUs associated with beneficial bacteria (e.g. *Faecalibacterium*) in this cohort. In addition, we found the increase of some OTUs related to potential bacterial pathogens (e.g. *Escherichia\_Shigella*) in these long-living people. While it is still early to draw any causal



**Figure 3.** Two recent studies (from a Japanese cohort (a) and from a Chinese Jiangsu province cohort (b)) found greater gut microbiome diversity in the long-living cohorts than in the younger cohorts.

relationships between gut microbiota and healthy aging, several recent studies have used animal models to show the causality of gut microbiome and age-related phenotypes. Smith et al showed that recolonizing the gut of middle-age fishes with bacteria from young donors led to life span extension and delayed behavioral decline.<sup>26</sup> In another mouse model study, Thevaranjan and colleagues showed that co-housing germ-free mice with old conventionally raised mice increased pro-inflammatory cytokines in the blood, such phenotype was not observed in these germ-free mice when co-housed with young mice.<sup>27</sup> Further studies using multi-omics approaches and animal models by fecal microbiota transplantation from young, elderly and long-living people will provide insights into the functions of the gut microbiome in healthy aging. Nevertheless, our study suggests that maintaining a diverse and balanced gut microbiome might contribute to healthy aging and provide a modulation target to promote healthy aging.

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