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### Correspondence

## Gut microbiota signatures of longevity

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An aging global population poses substantial challenges to society [1]. Centenarians are a model for healthy aging because they have reached the extreme limit of life by escaping, surviving, or delaying chronic diseases [2]. The genetics of centenarians have been extensively examined [3], but less is known about their gut microbiotas. Recently, Biagi *et al.* [4] characterized the gut microbiota in Italian centenarians and semi-supercentenarians. Here, we compare the gut microbiota of Chinese long-living people with younger age groups, and with the results from the Italian population [4], to identify gut-microbial signatures of healthy aging.

A total of 168 individuals (Dataset 1) from Dujiangyan and Ya'an, Sichuan province, China were enrolled in this study and assigned into two groups: a long-living group (≥90 years old), including 67 healthy centenarians and nonagenarians, and a younger age group, including 54 elderly and 47 young adults (Table S1). Gut microbiotas were characterized by sequencing the V4–V5 region of the 16S rRNA gene using the Illumina MiSeq platform.

Random Forest, a robust machinelearning technique that accounts for the nonlinear relationships and dependencies among microbiota features, was used to identify microbial signatures that best differentiate between the long-living and younger groups. We included alpha-diversity measures and the relative abundance of the top 500 operational taxonomic units (OTUs), accounting for 99.7% of the sequences as inputs (features), in the Random-Forest model. Each feature was assigned an importance score (mean decrease accuracy, MDA) based on the increase in error caused by removing that feature from the predictors. Features were ranked by their importance scores and those with an MDA>3 are considered highly predictive in this study. Interestingly, alpha diversities were among the top



#### Figure 1. Gut-microbiota signatures of longevity.

(A) Top 50 features from the combined Chinese and Italian dataset (Dataset 3) that differentiate long-living people from others in the Chinese population as revealed by Random Forest. A single-letter abbreviation for each phylum is given after the OTU#. The family and genus classifications by the Ribosomal Database Project (RDP) are also provided. Each feature (bacterial OTU or alpha diversity) was assigned an importance score (mean decrease accuracy). Stars denote the 11 features shared by the Chinese and Italian studies. (B,C) Box plots of (B) expected (Chao index) and (C) observed OTUs. (D–G) Relative abundance of OTUs, when present, labeled in yellow in panel A in the long-living (brown boxes) and young (green boxes) people in the Chinese and Italian studies. OTU175 (unclassified by the RDP) was classified as 'Christensenellaceae' by the Greengenes database. Abbreviations: P, Proteobacteria; F, Firmicutes; B, Bacteroidetes; V, Verrucomicrobia; A, Actinobacteria; CL, Chinese long-living group; CY, Chinese young group; IL, Italian long-living group; IY, Italian young group.



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features that distinguished long-living people from younger ones (Figure S1A). Greater community richness (number of taxa), including both expected (Chao) and observed numbers of OTUs, were observed in the long-living group (Figure S1B,C;  $p \le 0.001$ ). Although not listed as one of the top predictors, community diversity (Shannon index) was also greater in the long-living group than in the younger group (Figure S1D; p = 0.012).

To validate this discovery, we reanalyzed the Italian dataset (Dataset 2) [4]. Consistently, alpha-diversity measures (observed OTUs and Chao) were also listed as the top predictors of longevity in the Italian group (Figure S1E) and were greater in the long-living group than in the younger age group (Figure S1F,G; p = 0.028 and p = 0.058, respectively). In addition to the greater alpha diversity in the long-living group, several bacterial taxa were top predictors in both studies.

To determine if these bacteria are the same OTUs, we combined the two datasets (Dataset 3) and re-ran the analytical pipeline. Not surprisingly, significant differences in community membership and structures between the Italian and Chinese long-living groups were observed (Figure S1H,I). Furthermore, the most predictive OTUs also differed between the Chinese (OTU314, Pseudomonas, Figure 1A) and the Italian (OTU012, unclassified Ruminococcaceae, Figure S1J) datasets. Such differences could be attributed to many factors, such as geography, host genetics, diets, and DNA-extraction methods.

Despite these differences in the overall community structures, common features that discriminate long-living from young people were identified in both groups. Among the top 50 features that differentiate the Chinese long-living people from the younger groups, 11 were also listed as the top 50 in the Italian dataset, including community richness (Chao index and observed OTUs), members of Blautia (OTU014), Clostridium XIVa (OTU173), Faecalibacterium (OTU003), Escherichia\_Shigella (OTU005), unclassified Lachnospiraceae (OTU043, 028 and 282), Ruminococcaceae (OTU018), and Erysipelotrichaceae (OTU158) (Figures 1A and S1J).

OTUs enriched in the long-living groups in both cohorts, including members of the Clostridium cluster XIVa, Ruminococcaceae, Akkermansia and Christensenellaceae, are potentially beneficial bacteria. Clostridium cluster XIVa includes many genera involved in the production of short-chain fatty acids, which play important roles in gut homeostasis [5]. Several OTUs in this cluster were enriched in both the Chinese and Italian long-living groups, with OTU173 ranked as the top feature in both studies. Akkermansia and Christensenellaceae are linked to bodymass index, immunomodulation and healthy homeostasis [4,6].

In summary, our data support the idea that long-living people can be used to study the relationship between gut microbiota and healthy aging. Community diversity has been used as a marker for the health status of gut microbiota, with higher diversity associated with health and lower diversity associated with different diseases [7]. Recent studies revealed a negative correlation between community diversity and frailty in the elderly [8,9]. The higher gut microbial diversity and enrichment of several potentially beneficial bacterial taxa in long-living people from two independent studies reveal a healthier gut microbiota in this special group of people and indicate a link between healthy aging and gut microbiota.

Despite extensive studies on human genetics, limited progress has been made to link genetics with longevity [10]. Given its importance in health and disease, gut microbiota represents a new avenue in healthyaging studies. Although it is not clear whether the greater community diversity and enriched beneficial bacteria are the cause or effect of healthy aging, these features serve as microbial signatures of longevity and suggest potential targets to promote healthy aging.

#### SUPPLEMENTAL INFORMATION

Supplemental information includes experimental procedures, one figure, one table, author contributions, and supplemental references, and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.08.015.

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