Ageing-related bacterial features in different segments of the mice gastrointestinal tract

Qi Zhou

Beijing Hospital National center of Gerontology

Liang Sun (✉ sunbmu@foxmail.com)

Beijing Hospital National center of Gerontology

Linfang Li

Chinese Academy of Medical Science

Huan Gong

Beijing Hospital

Ying Zhang

Peking University Fifth School of Clinical Medicine

Chen Chen

Beijing Hospital

Jian Li

Beijing Hospital

Ze Yang

Beijing Hospital

Research article

Keywords: Healthy ageing, Gut microbiota, 16S rRNA sequencing, Gastrointestinal tract, mice

Posted Date: August 22nd, 2019

DOI: <https://doi.org/10.21203/rs.2.13401/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Ageing is associated with alternations of gastrointestinal (GI) microbiota according to metagenome sequencing. However, the most commonly used sequencing samples were from feces, therefore it remains unknown how the upper gastrointestinal microbiota changes with age and to what extent the fecal can represent the gastrointestinal microbiota. To investigate associations between the microbiota of whole GI tract and ageing, we compared microbial diversity and composition of six GI segments in different phenotypes with a mouse model. Results: Microbial α and β diversity were significantly different between the upper and lower GI tract. The jejunum and ileum samples had significantly lower phylogenetic diversity than large intestine and stomach did ($P < 0.01$). About 22.9% core OTUs ($n=80$) were shared by the whole GI tract, and fecal represented significantly higher microbiota with content from large intestine than content from upper GI tract (82.7% vs. 65.2%, $P < 0.001$). *Sutterella*, *Aggregatibacter*, *Lactococcus*, *Lactobacillus* and *Streptococcus* were significantly enriched in the upper GI tract, while 14 anaerobes such as *Ruminococcus* were significantly enriched in the lower GI tract ($P < 0.05$). The elderly mice had the significant microbial dissimilarity (both in α and β -diversity) with the young- and middle-aged ones. These differences were dependent on GI segments; especially in the lower GI tract, more obvious variations were found. However, the age-associated change was smaller when it compared with the high-fat diet treated mice. Conclusion: The GI microbiota was gradually changed with age and the changes were affected by GI segments. The microbial interactions with host motivate future studies exploring the specified GI microbiota interventions of disease. Keywords: Healthy ageing, Gut microbiota, 16S rRNA sequencing, Gastrointestinal tract, mice

Background

A balance in gastrointestinal (GI) microbial communities is crucial for host health maintenance in ageing[1]. Some ageing-associated diseases such as T2DM [2, 3], hypertension [4] and frailty [5, 6] have been hypothesized to be involved in imbalanced intestinal microbiota. This might because some gut microbial features have been proposed to be promoted by chronic low-grade inflammation, and to be able to nurture this process in turn [7]. The age-related changes in the gut microbiota including a decline in microbiota diversity, a decrease of core species and an increase of subdominant species, a shift from saccharolytic bacteria to proteolytic bacteria, and an increase of opportunistic species and pathobionts [8], have been reported in several human studies. However, most samples in studies were from feces, therefore, little information was obtained on the microbial associations between different GI segments and ageing.

Limited studies characterized the microbial relationship among different human GI segments, even though microbiota interactions with upper GI tract is also considered important for the host. For instance, some probiotics such as *Lactobacillus rhamnosus* and *Lactobacillus plantarum* are capable to reduce small intestinal injury induced by intraepithelial lymphocytes after Toll-like receptor 3 activation [9, 10]. Moreover, dysbiosis of gut microbiota could also be induced by ectopic colonization from the upper GI tracts, therefore, caused many diseases. For example, increased levels of microbes of oral origin have

been reported in the gut microbiota of patients with HIV infection [11] or liver cirrhosis [12]. *Klebsiella* strains from the mouth, resistant to multiple antibiotics, tend to colonize when the intestinal microbiota is dysbiosis, and could elicit a severe gut inflammation in the context of a genetically susceptible host [13]. Over-representation of intestinal commensal bacteria and reduced *Helicobacter* abundance was considered the dysbiosis of gastric microbiota; it could be used as a discriminant factor in distinguishing gastric carcinoma from chronic gastritis [14]. However, more work is required to gain detailed information about microbial changes among the whole GI tract and their associated impact with ageing.

Human gut microbiota was easily influenced by many factors such as diet, traveling and antibiotic [15], therein lies the problem that strong heterogeneities were always found in human especially the elderly intestinal microbial studies. Animal models were still most commonly used to study gut microbiota related diseases. So, in current study, we used C57BL/6 mice as a model to describe the relationship between GI tract and host. We firstly compared the microbiota differences along six GI segments including the stomach, jejunum, ileum, cecum, colon and feces by using 16S rRNA sequencing. Secondly, we investigated the age-related microbiota and how it was affected by GI segments. Finally, high fat feeding mice were used as a reference group to explore difference made separately by diet and ageing.

Methods

Study design

All animal procedures used in the present study were conducted in accordance with good animal practices as defined by the laboratory animal use license (Certificate No. SYXK (JING) 2011–0002). All work with mice was approved and supervised by approved by the Institutional Animal Care and Use Committee of the School of Life Science, Beijing Normal University. All mice and their diet were bought from Viewsolid Biotech company of China. The mice were housed in a standard animal laboratory with a 12 h light-dark cycle and were fed with a commercial diet in strict accordance with animal ethical standards. All mice used for GI content collection were euthanized by rapid decapitation at the endpoint.

Twelve male C57BL/6 mice were randomly divided into four groups: (1) Young-aged group (Ycon, 16 weeks) with a standard diet, (2) Middle-aged group (Mcon, 32 weeks) with a standard diet, (3) Elderly group (Econ, 72 weeks) with a standard diet, (4) Middle-aged group that have been fed with 45% high-fat diet for 14 weeks (Mhfd, 32 weeks). Commercial mouse chow and water were autoclaved before use. The contents from the stomach, jejunum, ileum, cecum and colon were sampled. Feces were collected the day when mice were euthanized. Samples were firstly collected into a tube filled with 1ml RNeasy lysis buffer (Qiagen, Germany) and then were stored at -80°C until DNA extraction.

16S rRNA sequencing and bioinformatics analysis of the sequencing data

Total DNA was extracted by the InviMAGE stool DNA kit (Invitex, Germany) following the manufacturer's introduction. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primers of 314F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The purified amplicons were pooled and sequenced on the HiSeq2500 (Illumina, San Diego, California, USA) using paired-end 300bp reads. Ends of forward and reverse reads were truncated at the base with a Phred quality score less than 20. Reads were merged when they are longer than 399bp and their overlap was more than 50-bp with the error was less than 0.1. Operational taxonomic units (OTUs) were clustered at the cutoff of 97% using the USEARCH v.7.0.1090 pipeline. Details of the protocol could be found at the website "http://drive5.com/usearch/manual/uparse_pipeline.html". OTUs were annotation using Greengene database v2013.5.99 by RDP classifier v2.2. α and β diversity were performed with QIIME v1.80 [43].

Statistical analysis

Alpha diversity index between groups was analyzed using two-way Student's t-test (Ycon, Mcon, Mhfd and Econ group) or Wilcoxon single rank test (GI segments). Changes in bacterial relative abundance were compared using Kruskal-Wallis rank test in more than three groups and using Wilcoxon single rank test in two groups. Permutational multivariate analysis of variance (PERMANOVA) and principle coordination analysis (PCoA) were used to compare the β diversity between biological groups. Multiple linear regression and K-Nearest Neighbor (KNN) model (4-fold cross-validation) were separately used to identify differential taxa and to test the ability in bio-group classification with GI microbiota. All the statistical analysis were performed by R language (R×64 3.5.1).

Results

Bacterial features in the different mouse GI segments

A total of 3,550,520 valid reads with a mean number of 26,496 tags for each sample were obtained from 68 samples of 12 mice (Table S1). All the sample rarefaction curves tended to approach the saturation plateau and finally, 15,180 OTUs were obtained by using 97% homology cut-off value (Fig. S1, Table S1). By investigating the microbial α diversity (observed species, Chao index, Ace index and Shannon index) of different GI segments, we found that it changed following a "U" shape (Fig. 1a, Fig. S2). Generally, the α diversity significantly lower in jejunal and ileal samples than that from the stomach, cecum, colon, and feces (Wilcoxon test, $P < 0.01$).

We next compared β diversity of microbes in the six parts of the GI tract according to the Bray-Curtis similarity of the genus. Bacteria displayed volatility in upper and lower GI tract (Fig. 1b). The lower GI tract including cecum, colon and feces shared similar microbial composition (Bray-Curtis similarity ≥ 0.8) and the same pattern was also observed in upper GI bacteria (Bray-Curtis similarity ≥ 0.6). However, the

similarity was decreased when it was calculated between the upper and lower GI tract (Bray-Curtis similarity ≤ 0.6).

Moreover, there were 350 unique core OTUs (existing in more than 50% samples per GI segment) were observed in the whole GI tract. 276, 107, 106, 247, 274 and 251 core OTUs were found, respectively, in content from stomach, jejunum, ileum, cecum, colon and feces (Fig. 1c, red bars). About 22.9% core OTUs (n=80) were shared by the whole GI tract, and 40% (n=140) core OTUs were shared by lower GI tract and stomach, but not by jejunum and ileum (Fig. 1c, black bars).

To investigate how much GI microbiota could be represented by feces, we explored the Jaccard distances of OTUs between in fecal and the other segments (Fig. 1d). Feces shared about 82.7% OTUs with the lower GI tract, while it shared about 65.2% OTUs with the upper GI tract (Wilcoxon test, $P < 0.001$). The most similar GI segments with feces of microbial composition were colon who shared 88.74% OTUs, followed by cecum (76.7%), stomach (69.9%), jejunum (65.5%) and ileum (60.3%).

Finally, we explored the microbial compositional differences among GI segments. Taxonomically, 14 phyla were observed. The most abundant one was Firmicutes (51.27%) followed by Bacteroidetes (35.18%), Proteobacteria (9.17%), Actinobacteria (1.87%) and the others (Fig. S3a). Of all the phyla, only Cyanobacteria showed significant difference among the six parts of the GI tract (Kruskal-Wallis test, $P < 0.001$). Its abundance was lower in jejunal and ileal than the other GI segments in mice of all ages (Fig. S3b). It is worth noting that phylum variation in different GI tract was affected by aging and high-fat diet intervention. For example, we found that the older mice and the high-fat feeding ones shared the same trend in Firmicutes variation among the six GI segments: the Firmicutes abundance in jejunal and ileal was higher than the other parts, indicating that the Firmicute might be less susceptible to the jejunal environment when the mice became older or fat (Fig. 2a). However, our results showed that Bacteroidetes and Proteobacteria revealed a antagonistic trend with Firmicute among the whole GI tract in all the biological groups. It is different with Cyanobacteria whose change rule was similar in various age groups. The relative abundance of Cyanobacteria was increased from jejunal to colon (Fig. S3c). In genus level, there were 19 genera that were differently composed in among the six GI tracts according to the Kruskal-Wallis test with the cutoff P value < 0.05 (Table S2). They were clustered into two groups (Cluster1 and Cluster2) in first of which *Sutterella*, *Aggregatibacter*, *Lactococcus*, *Lactobacillus* and *Streptococcus* were significantly enriched in the upper GI tract, while anaerobes such as *Ruminococcus* were significantly enriched in the lower GI tract in Cluster2 (Fig. 2c).

The bacterial community was significantly different in the elderly mice.

Four diversity indexes including the number of observed species, Chao index, ACE index and Shannon index were compared among three age groups (Fig. 3a and Fig. S4). The observed species, Chao index and ACE index, were significantly declined in elderly mice, but this trend was only observed in the lower GI

tract (Student's t-test, $P < 0.05$). This result might indicate that lower GI microbes were more likely to be affected by ageing, compared with the upper GI bacteria.

The whole GI microbiota composition changed significantly with ageing according to PERMANOVA analysis (permutations = 999, method="bray", $P < 0.001$). When OTUs based Bray-Curtis distance was constructed to compare the GI microbial β diversity between the young-aged, middle-aged and the elderly mice, it was found that the distances between the elderly group and non-elderly group (the young-aged and middle-aged group) were larger than that between the young-aged and middle-aged group (Wilcoxon test, $P < 0.001$). However, microbial composition in the stomach had no significant differences among the three age groups (Fig. 3b).

To further investigate the taxonomic changes with age, we compared the microbial relative abundance between the three age groups in both phylum level and genus level. Key taxon selection was performed according to Kruskal-Wallis rank test, followed by a multiple linear regression model in which the age-group was treated as an ordinal categorical variable to enhance its robustness (Table S3, Table S4). Eventually, 5 phyla and 20 genera were observed to change marginally (Kruskal-Wallis rank test, $P < 0.1$) from young-aged groups to the elderly mice (Table S3, Fig. 3c). There were 3 genera, namely *Allobactulum*, *Sutterella* *Lactobacillus*, that clearly changed with ageing in all the GI segments. Their relative abundance was higher in upper GI tract, especially in jejunum and ileum than it in the lower GI tract (Fig. 3d). Some of ageing associated changes were also affected by GI segments. For instance, the increment of Firmicute in elderly as well as the depletion of both Proteobacteria and Bacteroidetes was more likely to be observed in ileum and jejunum than that in the other GI segments (Fig. 3c). But *Coprococcus* decrement and *Flexispira* increment in elderly mice were only observed in the cecum, colon and feces (Fig. 3d). Similarly, the abundance of *Streptococcus* was higher in elderly mice than that in the other age groups and it was only observed in the upper GI tract (Fig. 3d).

GI microbiota gradually changed with age when it was compared to high-fat diet treatment.

It has been reported that there is no chronological threshold or age at which the composition of the microbiota suddenly alters [16], thus, it was necessary to understand how much differences it would make separately by ageing and by other conditions such as lifestyle, disease and diet. Here, we choose high-fat diet mice as a reference group to compare the diet cased GI changes with the ones made by ageing. Cluster as well as principle coordination analysis (PCoA), based on Bray-Curtis distance of OTUs, showed that high-fat feeding mice were clustered separately from the other groups (Fig. 4ab). The intra-dissimilarity which calculated between each two of normal feeding mice was significantly lower than the inter-distance which calculated between high-fat feeding mice and normal feeding mice (Fig. 4c, Wilcoxon test, $P < 0.001$). In addition, we also constructed a KNN model with genus composition to classify biological groups. It was more difficult to pick one of the three age-related groups (Ycon, Mcon and Econ) than the high-fat feeding ones (Mhfd), because the accuracy to separate high-fat feeding mice from the others reached 0.94, whereas the highest accuracy to classify the three age-related groups were

only 0.65 (Fig. S5). To sum up, these results suggested the healthy aging-related microbial changes were moderate, and it was not as intense as the diet/disease associated ones.

We then investigated the high-fat feeding associated genera in the GI tract (Table S4). There are 22 marginally different genera were found between the high-fat feeding and normal feeding groups (Wilcoxon test, $P < 0.1$). Most genera were non-overlapped with the age-related ones, and the overlapped 4 genera including *AF12*, *Allobaculum*, *Bifidobacterium*, *Bilophila*, *Butyricicoccus* were independently associated with different GI segments (Fig. 4d).

Discussion

By modulating ageing-related changes in innate immunity and cognitive function, the gut microbiota was reported to affect health in older individuals [16]. However, many of these hypotheses were investigated by fecal samples, and how the microbiota in upper GI tract interacts with healthy ageing remain out of reach. Moreover, it is pivotal to study to what extent the feces could represent the microbiota upper GI tract since it is one of the most frequently used invasive sampling techniques. In the present study, we used C57BL/6 mice to explore microbial features among six GI segments and their microbial relationship with ageing.

The overall taxonomic groups of this study were similar with previous findings that Firmicutes, Bacteroidetes and Proteobacteria were the most three abundant phyla [17, 18] in the GI tract. However, who is the most dominant phyla in mice is confused for the various diet, lifestyles and other reasons. For example, Chang *et al* [19] found Firmicutes was more than Bacteroidetes in obese mice, whereas Caricilli *et al* [20] showed an opposite result in TLR2 knockout mice. Basing on the fecal samples, a dramatic shift from Firmicutes that dominated the gut microbiota of young life toward Bacteroidetes in the elderly was observed both in mice [18] and humans [21]. This shift was also observed in our samples from the lower GI tract (microbiota from cecum, colon and feces), although the changes were not significant. In contrast, Firmicute became the dominate microbiota in the upper GI tract in elderly mice was easily found in this study. This may portend a different mechanism of microbial interactions with different GI segments. In ileostomy samples from humans the small intestine was found a high enrichment of *Clostridium* spp. which is different in our study, and there were a certain members of Proteobacteria [22] which we only observed in high-fat feeding and the elderly mice.

The traditional concept holds that bacterial along the mammalian GI tract was increased from the stomach to feces, because of the harsh conditions in upper GI tract, such as lower PH in the stomach and more antimicrobial peptides produced by Paneth cells of the small intestine[23-25]. The bacteria ranges from 100-1000 per ml in the stomach to $\sim 10^5$ per ml in the upper small intestine and up to 10^{12} per ml in the colon [25, 26]. This concept was partially supported by our study that the microbial α diversity was increased from small intestine to large intestine. The greatest density of bacteria in cecum, which has

been suggested to explain the higher prevalence of tumors compared with the adjacent small intestine[27], was also observed along the whole GI tract in our study. Of note, we also found a greater number of species (observed species of alpha diversity) in the stomach where the highly acid environment was not suitable for many bacteria. It may result from the food, water and the outer environment such as fur, and most of them were considered as “transient microbiota”. In addition, it was possible that these “transient microbiota” came from the saliva in which contained many bacteria like *Streptococcus*, *Neisseria*, *Veillonella*, etc [28]. In fact, high microbial diversity in the stomach has been also detected in human, horse and the other mice [29-31]. In microbial composition, although many species were seeded from the food we eat or the saliva we swallowed [32], only 22.9% of core OTUs were actively transferred from stomach to colon. The overlapped OTUs number in human GI tract were even less than the mice that only ~ 0.1 % OTUs were shared from the human mouth to the colon [33]. The harsh condition of both stomach and small intestine is essential to keep the host healthy because it enriched some beneficial bacteria such as *Lactobacillus* who is abundant in jejunum and ileum in human [26] and mice. However, this function was affected by the host, since the microbial composition was different in pigs that *Escherichia*, *Acinetobacter* and *Enterobacteriaceae* dominated in small intestine [34]. Additionally, we found compositional similarities in human and mice that *Streptococcus* (67% in human jejunal biopsies) and *Lactobacillus* were dominated in the small intestine [35, 36].

Although the gut microbiome is changing gradually with age [16], we observed a significant variation in elderly mice in both alpha diversity and β diversity. The β diversity between the older mice and middle ones was larger than the diversity between middle- and young-aged mice in the current study. The α diversity was significantly lower in elderly mice. Studies exploring microbial changes with ageing have pointed out that the differences in the gut microbiota composition between young adults and the elderly were smaller than the differences observed between the elderly and centenarians [37]. These results from both mice and humans suggested that gut microbiota is relatively stable in younger adulthood and it will become more unsteady after entering older age. Recently, researchers studying the gut bacteria of thousands of people found the microbiome could be used as a biological age clock to predict the age according to machine learning [38]. Research on age-dependent patterns of gut microbial diversity in human adults found a strong association between age and alpha diversity was kept until 40 years old [39]. However, at which age the gut microbiome composition will significantly change has not been clearly mentioned in all the previous researches. This is because the microbial heterogeneous is very large in the elderly, and the microbiome is easily influenced by factors such as nutrition, lifestyle, ethnic origins and gender, etc. [40-42]. Microbiota in which GI segments were most likely to be used for disease and biological age identification is still unclear. In this study, we found that except in the stomach, microbiota in every segment of the GI tract could be used to separate the elderly from the young- and middle-aged mice. According to the comparison of the microbiome in the whole GI tract, we found α diversity in the lower GI tract was more easily affected by ageing. This could result from the neutral PH and low decreased immunogenicity of lower GI. The suitable environment for escaped bacteria from the upper GI tract enlarged the microbial signals among three age groups. There were no-significant compositional differences observed in stomach among three age groups. It might be related to the high

percentage of transient microbiota from the mouth and this similarity may largely explain the microbial homogeneity of diet.

High fat diet feeding mice were used as a reference group in this study to investigate how much microbial changes would cause by the diet-related disease when it compared with the age-related changes. The healthy ageing associated microbial change was considered the baseline characterization that should be adjusted as a confounder in many clinical studies. Here, we found small microbial interruption with ageing, indicating the possibility to use gut microbiome as a tool for disease identification. The same trend was observed in previous research that microbiome in high-fat feeding and low-fat feeding mice were clustered separately independent of the age (62 weeks and 83 weeks) [18]. However, microbiota at the extreme age of mice (141 weeks, both high-fat and low-fat diet) clustered separately with the other two groups. This might indicate a significant difference in longevity samples.

There are some limitations in our study: the mouse number in each biological group was small (Three biological repeats per group). Thus, we used more than two strategies to get robust findings. For example, we first performed both the Kruskal-Wallis rank test and a linear regression model adjusted GI segments to co-select ageing-related taxa. Second, the similarity with published results further proved our concept. This is the first time that microbiota of the whole GI tract was compared in different age groups and the result needs to be tested with additional researches.

Conclusions

Although previous microbiome studies in both humans and mice have shown changes with age, the association between the microbiota in the upper GI tract and ageing remains unclear, because most previous results were obtained by fecal samples. Here, by using a mouse model, we described the taxonomic overlaps and differences among the whole GI microbiota, and provide evidence that microbiota in both the upper and lower GI tract is closely related with ageing. In addition, we observe that the GI microbiota was gradually changed with age when it is compared with the high-fat feeding treatment. Together, this study furthers our knowledge of dynamics of mouse microbiota in the whole GI tract with healthy ageing. It encourages us a new focus on the variable microbial ecosystem in different location of the GI tract among several biological phenotypes.

Abbreviations

GI: Gastrointestinal; OTUs: Operational taxonomic units; PERMANOVA: Permutational multivariate analysis of variance; PCoA: Principle coordination analysis; KNN: K-Nearest Neighbor; Mhfd, high fat feeding middle-aged mice; Econ, Elderly mice with normal diet; Mcon, Middle-aged mice with normal diet; Ycon, Young-aged mice with normal diet.

Declarations

Acknowledgments

Not applicable

Funding

The mice purchase and raising were supported by the National Scientific Foundation of P. R. China (no. 81571385 and 91849132). DNA extraction and sequencing were funded by Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2018RC330003), CAMS Innovation Fund for Medical Sciences (2018-I2M-1-002), the Beijing Hospital Nova Project (BJ-2018-139) and the Doctoral Scientific Research Foundation (no. bj-2018-024). The funding body did not have a role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

QZ and LS conceived the study; HG, YZ and CC performed the experiments; QZ performed the statistical analysis and wrote the initial draft of the manuscript; LL, JL and ZY revised the manuscript; all authors read and approved the final manuscript.

Availability of data and materials

The phylum and genus relative abundance were contained in Additional file 1. The raw data of the sequencing during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal procedures used in the present study were conducted in accordance with good animal practices as defined by the laboratory animal use license (Certificate No. SYXK (JING) 2011–0002). The animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the School of Life Science, Beijing Normal University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. An R, Wilms E, Masclee AAM, Smidt H, Jonkers D: Age-dependent changes in GI physiology and microbiota: Time to reconsider? *Gut*. 2018;67(12):gutjnl-2017-315542.
2. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D et al: A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; 490:55.
3. Karlsson FH, Valentina T, Intawat N, G?Ran BM, Carl Johan B, Bj?Rn F, Jens N, Fredrik BC: Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*. 2013;498(7452):99-103.
4. Jing L, Zhao F, Wang Y, Chen J, Jie T, Gang T, Wu S, Liu W, Cui Q, Geng B: Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* 2017;5(1):14.
5. Tongeren SP, Van, Slaets JPJ, Harmsen HJM, Welling GW: Fecal microbiota composition and frailty. *Appl Environ Microb*. 2005;71(10):6438-6442.
6. Jackson MA, Jeffery IB, Beaumont M, Bell JT, Clark AG, Ley RE, O'Toole PW, Spector TD, Steves CJ: Signatures of early frailty in the gut microbiota. *Genome Med*. 2016;8(1):21.
7. Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkila J, Monti D, Satokari R, Franceschi C: Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians. *Plos One* 2010;5(5):e10667.
8. Vaiserman AM, Koliada AK, Marotta F: Gut microbiota: A player in aging and a target for anti-aging intervention. *Ageing Res Rev*. 2017;35(Complete):36.
9. Tada A, Zelaya H, Clua P, Salva S, Alvarez S, Kitazawa H, Villena J: Immunobiotic *Lactobacillus* strains reduce small intestinal injury induced by intraepithelial lymphocytes after Toll-like receptor 3 activation. *Inflammation Res*. 2016; 65(10):771-783.
10. Villena J, Chiba E, Vizoso-Pinto M, Tomosada Y, Takahashi T, Ishizuka T, Aso H, Salva S, Alvarez S, Kitazawa H: Immunobiotic *Lactobacillus rhamnosus* strains differentially modulate antiviral immune response in porcine intestinal epithelial and antigen presenting cells. *BMC Microbiol*. 2014;14(1):126.
11. Lozupone C, Li M, Campbell T, Flores S, Linderman D, Gebert M, Knight R, Fontenot A, Palmer B: Alterations in the Gut Microbiota Associated with HIV-1 Infection. *Cell Host Microbe*. 2014;14(4):329-339.
12. Nan Q, Fengling Y, Ang L, Edi P, Yanfei C, Li S, Jing G, Emmanuelle LC, Jian Y, Lingjiao W: Alterations of the human gut microbiome in liver cirrhosis. *Nature*. 2014;513(7516):59-64.
13. Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, Kiguchi Y, Yasuma K, Watanabe E, Tanoue T et al: Ectopic colonization of oral bacteria in the intestine drives T_H1 cell induction and inflammation. *Science*. 2017;358(6361):359.

14. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, Costa JL, Carneiro F, Machado JC, Figueiredo C: Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. *Gut*. 2017;67(2):gutjnl-2017-314205.
15. Brüssow H: How stable is the human gut microbiota? And why this question matters. *Environ Microbiol*. 2016;18(9):2779-2783.
16. O'Toole PW, Jeffery IB: Gut microbiota and aging. *Science* 2015, 350(6265):1214-1215.
17. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R: Evolution of Mammals and Their Gut Microbes. 2008;320(5883):1647-1651.
18. Zhang C, Li S, Yang L, Huang P, Zhao L: Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nat Commun*. 2011;4(1):2163.
19. Chang CJ, Lin CS, Lu CC, Martel J, Ko YF, Ojcius D, Tseng SF, Wu TR, Margaret Chen Y-Y, D Young J et al: *Ganoderma lucidum* reduces obesity in mice by modulating the composition of the gut microbiota, *Nat Commun*. 2005;6:7849.
20. Caricilli AM, Picardi PK, de Abreu LL, Ueno M, Prada PO, Ropelle ER, Hirabara SM, Castoldi n, Vieira P, Camara NOS: Gut Microbiota Is a Key Modulator of Insulin Resistance in TLR 2 Knockout Mice. *Plos Biology*. 2011;9(12):e1001212.
21. Claesson MJ, Cusack S, O'Sullivan O, Greenediniz R, Weerd HD, Flannery E, Marchesi JR, Falush D, Dinan T, Fitzgerald G: Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci USA* 2011;108 Suppl 1(11):4586-4591.
22. Zoetendal EG, Raes J, Van dBB, Arumugam M, Booijink CC, Troost FJ, Bork P, Wels M, de Vos WM, Kleerebezem M: The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *The ISME Journal*. 2012; 6(7):1415-1426.
23. Ouellette, J A: Paneth cells and innate mucosal immunity. *Curr Opin Gastroen*. 2010;26(6):547-553.
24. Clevers HC, Bevins CL: Paneth Cells: Maestros of the Small Intestinal Crypts. *Annu Rev Physiol*. 2013;75(1):289-311.
25. Donaldson GP, Lee SM, Mazmanian SK: Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*. 2016;14(1):20-32.
26. Mowat AM, Agace WW: Regional specialization within the intestinal immune system. *Nat Rev Immunol*. 2014;14(10):667.
27. Bongers G, Pacer ME, Geraldino TH, Chen L, He Z, Hashimoto D, Furtado GC, Ochando J, Kelley KA, Clemente JC: Interplay of host microbiota, genetic perturbations, and inflammation promotes local development of intestinal neoplasms in mice. *J Exp Med*. 2014;211(3):457-472.
28. Ogawa T, Hirose Y, Honda-Ogawa M, Sugimoto M, Sasaki S, Kibi M, Kawabata S, Ikebe K, Maeda Y: Composition of salivary microbiota in elderly subjects. *Sci Rep*. 2018;8(1):414.
29. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, Perezperez G, Blaser MJ, Relman DA: Molecular analysis of the bacterial microbiota in the human stomach. *PNAS*. 2006;103(3):732-737.

30. Perkins GA, den Bakker HC, Burton AJ, Erb HN, McDonough SP, McDonough PL, Parker J, Rosenthal RL, Wiedmann M, Dowd SE: Equine Stomachs Harbor an Abundant and Diverse Mucosal Microbiota. *Appl Environ Microb*. 2012, 78(8):2522-2532.
31. Shenghua G, Dandan C, Jin-Na Z, Xiaoman L, Kun W, Li-Ping D, Yong N, Xiao-Lei W: Bacterial community mapping of the mouse gastrointestinal tract. *Plos One* 2013, 8(10):e74957.
32. Brüssow, Harald: How stable is the human gut microbiota? And why this question matters. *Environ Microbiol*. 2016;18(9):2779-2783.
33. Stearns JC, Lynch MDJ, Senadheera DB, Tenenbaum HC, Goldberg MB, Cvitkovitch DG, Croitoru K, Morenoghagsieb G, Neufeld JD: Bacterial biogeography of the human digestive tract. *Sci Rep*. 2011;1(2840):170.
34. Zhao W, Yapeng W, Shuyun L, Jiaojiao H, Zhengxiao Z, Chuan H, Jinmei D, Jun W, Huijuan W, Weibing F: The Dynamic Distribution of Porcine Microbiota across Different Ages and Gastrointestinal Tract Segments. *Plos One* 2015, 10(2):e0117441.
35. Wang M, Ahrné S, Jeppsson B, Molin G: Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. *Fems Microbiol Ecol*. 2005;54(2):219-231.
36. Derrien M, Vlieg JETVH: Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends Microbiol*. 2015;23(6):354-366.
37. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turrone S, Consolandi C, Quercia S, Scurti M, Monti D: Gut Microbiota and Extreme Longevity. *Curr Bio*. 2016;26(11):1480-1485.
38. Galkin F, Aliper A, Putin E, Kuznetsov I, Gladyshev VN, Zhavoronkov A: Human microbiome aging clocks based on deep learning and tandem of permutation feature importance and accumulated local effects. *bioRxiv*. 2018:507780.
39. de la Cuesta-Zuluaga J, Kelley ST, Chen Y, Escobar JS, Mueller NT, Ley RE, McDonald D, Huang S, Swafford AD, Knight R et al: Age- and Sex-Dependent Patterns of Gut Microbial Diversity in Human Adults. *mSystems*. 2019;4(4):e00261-19
40. Mélanie D, Bouter KE, Andrei P, Evgeni L, Groen AK, Hilde H, Valentina T, Bakker GJ, Ilias A, Sara-Joan PS: Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat Med*. 2018;24:1526-1531
41. Tanya Y, Rey FE, Manary MJ, Indi T, Maria Gloria DB, Monica C, Magda M, Glida H, Baldassano RN, Anokhin AP: Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222-227.
42. de la Cuesta-Zuluaga J KS, Chen Y, Escobar JS, Mueller NT, Ley RE, McDonald D, Huang S, Swafford AD, Knight R, Thackray VG.: Age- and sex-dependent patterns of gut microbial diversity in human adults. *mSystems*. 2019;4(e0026):1-19.
43. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI et al: QIIME allows analysis of high-throughput community sequencing data. *Nat Mehtods*. 2010;7:335.

Figures

Figure 1

Differences of both microbiota diversity and composition among different GI segments. a α diversity of microbiota in GI tract (Chao index and Observed species). b Microbial similarity between each two parts of GI tract (Bray-Curtis similarity). c Core OTUs numbers that shared by microbiota of different GI segments. A core OTU were defined when it existed in more than 50% samples. d Microbial similarity between feces and the other GI parts (Jaccard similarity).

Figure 2

Comparison of relative abundances of gut microbiota profile in phylum level (a) and genus level (b). Mhfd, high fat feeding middle-aged mice. Econ, elderly mice with normal diet. Mcon, middle-aged mice with normal diet. Ycon, young-aged mice with normal diet. Error bar were shown by vertical lines.

Figure 3

Microbial diversity and compositional differences among three age groups. a Observed species and Chao index were compared among the three age related groups. b microbiota distances were compared between every two age related groups. Microbiota that marginally significantly different among three age related groups in phylum (c) and genus level (d). Mcon/M, middle-aged mice with normal diet. Ycon/Y, young-aged mice with normal diet. Econ/E, elderly mice with normal diet. Symbols indicate the significance of a Wilcoxon test between the elderly and non-elderly groups (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

Figure 4

Comparison of GI microbiota between age related and high fat feeding related groups. Cluster analysis (a) and PCoA (b) of samples from each biological group based on Bray-Curtis distance. c Comparison of intro-dissimilarity (Bray-Curtis distance between each two of normal feeding mice) and inter-dissimilarity (Bray-Curtis distance between high-fat feeding mice and normal feeding mice). d Comparison of high fat feeding related genera and ageing related genera. Mhfd, high fat feeding middle-aged mice. Econ, elderly mice with normal diet. Mcon, middle-aged mice with normal diet. Ycon, young-aged mice with normal diet. Error bar were shown by vertical lines.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS3.csv](#)
- [AdditionalFile1.xlsx](#)
- [ARRIVEGuidelinesChecklist.docx](#)
- [TableS4.csv](#)
- [TableS5.csv](#)
- [TableS2.csv](#)