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Mendelian randomization analyses support causal relationships between gut microbiome and longevity

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Abstract

Background Gut microbiome plays a significant role in longevity, and dysbiosis is indeed one of the hallmarks of aging. However, the causal relationship between gut microbiota and human longevity or aging remains elusive.

Methods Our study assessed the causal relationships between gut microbiome and longevity using Mendelian Randomization (MR). Summary statistics for the gut microbiome were obtained from four genome-wide association study (GWAS) meta-analysis of the MiBioGen consortium ($N=18,340$), Dutch Microbiome Project ($N=7738$), German individuals ($N=8956$), and Finland individuals ($N=5959$). Summary statistics for Longevity were obtained from Five GWAS meta-analysis, including Human healthspan ($N=300,447$), Longevity ($N=36,745$), Lifespans ($N=1,012,240$), Parental longevity ($N=389,166$), and Frailty (one of the primary aging-linked physiological hallmarks, $N=175,226$).

Results Our findings reveal several noteworthy associations, including a negative correlation between *Bacteroides massiliensis* and longevity, whereas the genus *Subdoligranulum* and *Alistipes*, as well as species *Alistipes senegalensis* and *Alistipes shahii*, exhibited positive associations with specific longevity traits. Moreover, the microbial pathway of coenzyme A biosynthesis I, pyruvate fermentation to acetate and lactate II, and pentose phosphate pathway exhibited positive associations with two or more traits linked to longevity. Conversely, the TCA cycle VIII (helicobacter) pathway consistently demonstrated a negative correlation with lifespan and parental longevity.

Conclusions Our findings of this MR study indicated many significant associations between gut microbiome and longevity. These microbial taxa and pathways may potentially play a protective role in promoting longevity or have a suppressive effect on lifespan.

Introduction

The increases in life expectancy were observed globally during the past 50 years [1, 2]. Previous studies have described growing evidence highlighting geographical disparities in life expectancy at county and sub-county levels [3], which may be associated with genetics [4, 5], environmental factors including intestinal microbiota [6] and socioeconomic factors [7]. Dysbiosis is one the twelve hallmarks of aging [8], and it contributes to multiple pathological conditions associated with age-related diseases, such as diabetes, hypertension, and Alzheimer disease [9, 10]. Age-related morbidities affect the quality and quantity of life, but the composition and function and within the intestinal microbiome as we age are not completely understood. Thus, identifying aging patterns within the gut microbiome

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could have clinical implications for both monitoring and modifying gut health to achieve healthy aging.

Several studies have characterized the gut microbiota in centenarians [7, 11–14], providing potential insight into gut microbial trajectories associated with aging. For example, *Akkermansia* was found to be enriched in the gut of centenarians [7, 11–14], while *Bacteroides* and *Faecalibacterium* were relatively depleted in centenarians [7, 13]. However, most observational research cannot infer the causal relationship between gut microbiota and human longevity or aging. Few mechanisms that link longevity- or aging-correlated pathophysiology with specific microbes or functional pathways have been identified [12, 15]. Some microbial pathways that regulate aging were discovered in invertebrates such as *Caenorhabditis elegans* (*C. elegans*) [16, 17] and drosophila [18], however, the genetics of aging is more complex in vertebrates and primates (including *Homo sapiens*) because of their specialized systems [19]. Population-scale human genome and metagenome sequencing projects combined with comprehensive data on lifespan and age-related diseases are opening new avenues to understand the genetics and microbiome involved in human aging.

In recent years, Mendelian randomization (MR) has attracted wide attention by inferring the causal relationship of modifiable exposures on an outcome such as disease status [20]. For example, a MR analysis indicated a potential causal effect of *Morganella* on major depressive disorder, implying that this bacterium may play a role in the metabolic modulation of health within the brain-gut axis [21]. Another study employed MR to infer causal relationships between the gut microbiome and metabolites, discovering a potential causal role of *Eubacterium rectale* in decreasing plasma levels of hydrogen sulfite-a toxin that affects cardiovascular function [22]. These studies uncover potential metabolic capabilities of gut microbes that exert an influence on human health. Therefore, this study assessed the causal relationships between the gut microbiome and longevity using MR. Previous microbiome-longevity studies utilizing MR analysis relied on a limited dataset [23, 24], which resulted in findings that were not comprehensive. Here, we have utilized the most comprehensive GWAS summary data, as of the present moment, pertaining to the gut microbiome and longevity. Our findings provide novel clues for understanding the roles of gut microbiota in longevity and aging development, which may lead to the development of microbiome-based therapies and personalized medicine approaches to delay aging and promote longevity.

Methods

Study design

Figure 1 outlines this study's overall design and data source. Briefly, a two-sample MR analysis was performed to verify causal links between gut microbiota and traits associated with longevity. It takes genetic variation (single nucleotide polymorphisms, SNPs) as the instrumental variables (IVs) to deduce the causal effect of the exposure (gut microbiota) on the outcome (longevity-related traits), thereby effectively circumventing the confounding biases often encountered in traditional epidemiological studies. Three MR assumptions are used for the unbiased estimation of possible causal relationships: (1) IVs are strongly associated with exposure (relevance); (2) IVs are independent of any confounding factors (independence); and (3) IVs affect the outcome only through the exposure (exclusion restriction) [25]. The genome-wide association study (GWAS) data source of longevity-related traits and gut microbiota has also been summarized in Table S1.

GWAS data of human healthspan, longevity, lifespans, parental longevity, and frailty

Five publicly available GWAS datasets of longevity-related traits were used in this study. The GWAS data of human healthspan consists of 300,477 British-ancestry individuals from the UK Biobank (UKB) [26]. The GWAS data of longevity was derived from 36,745 individuals of European ancestry in multiple studies, encompassing 11,262 cases and 25,483 controls, and the cases were individuals who lived to age above the 90th percentile or 99th percentile [5]. The GWAS data of lifespan consists of 1,012,240 European-ancestry individuals, including 512,047 mother and 500,193 father lifespans [27]. The GWAS data of parental longevity was collected for 389,166 UKB participants of European descent with data recorded on parents' current ages or parents' ages of death [28]. Pilling et al. identified all common genetic variants associated with longer parental lifespan, including 7 traits (mother's age at death, father's age at death, mother's attained age, father's attained age, combined parental age at death, combined parental attained age, and both parents in top 10%) [28]. Because one of the primary aging-linked physiological hallmarks is the onset of frailty [29], the GWAS data of frailty is also incorporated in our study, which included 175,226 individuals of European descent and used the frailty index (FI) to measure frailty [30].

GWAS data of gut microbiota

Genetic variants associated with the gut microbiome were obtained from four datasets. The first dataset was conducted by MiBioGen consortium, and integrated 16S

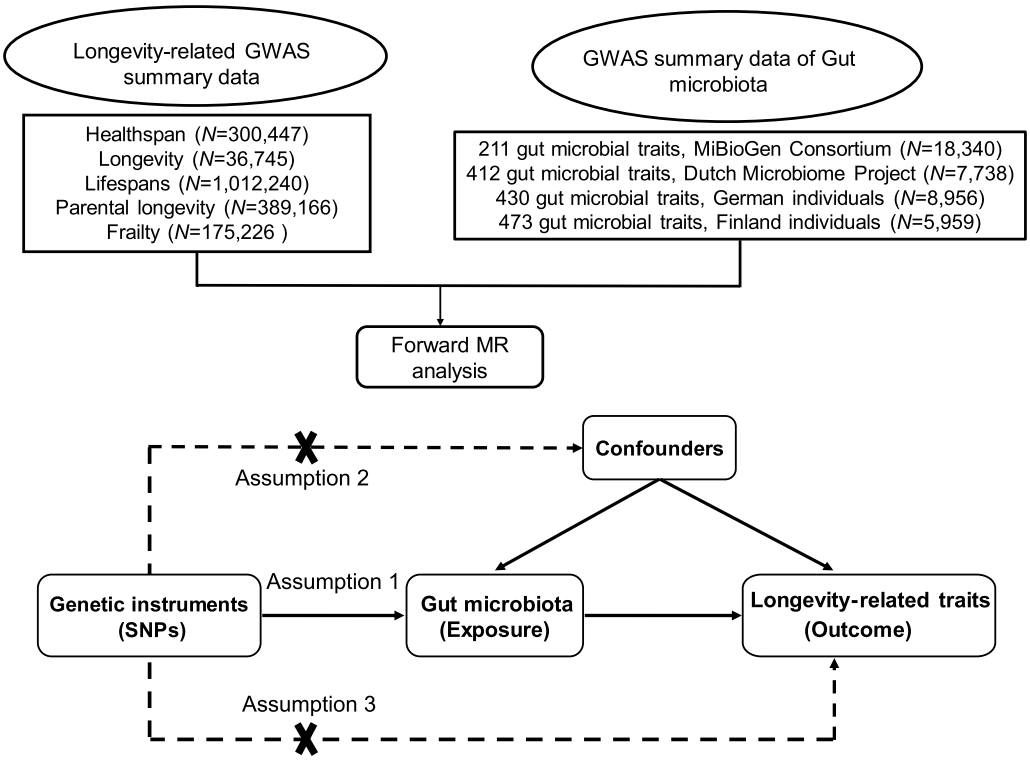


Fig. 1 The study design of the MR investigation pertaining to the associations between gut microbiota and longevity

rRNA gene sequencing profiles and genotyping data from 18,340 participants across 24 cohorts [31]. Most of participants had European ancestry ($N=13,266$). Both genetic data and gut microbiota data were incorporated, and association estimates for a total of 211 bacterial taxa were calculated. After excluding 15 taxa of bacteria without specific names (unknown family or genus), gut microbiota was divided into 196 bacterial taxa including 9 phyla, 16 classes, 20 orders, 32 families, and 119 genera. The second dataset was obtained from 7,738 participants of the Dutch Microbiome Project (DMP), and the gut microbiota was identified by shotgun metagenomic sequencing of stool samples [32]. It contained 207 taxa (105 of which are species) and 205 functional pathways that reflect the composition and activity of gut microbiota. The third dataset was obtained from 8956 German individuals by Ruhlemann et al. [33], which carried out a GWAS involving 430 taxa (from phylum to genus, examining the abundance and prevalence in fecal samples) that reflect the composition of gut microbiota. The fourth dataset was a large-scale population-based cohort of 5959 Finland individuals enrolled in the FINRISK 2002 (FR02) cohort, which carried out a GWAS involving 473 taxa (including species level, examining the abundance in stool) that provided insights into the composition of gut microbiota[21].

Selection of instrumental variables (IVs)

We extracted the gut microbiota taxa as exposure data, including 196 bacterial taxa from the MiBioGen consortium, 412 bacterial traits (207 taxa and 205 pathways) from the DMP, 430 taxa from 8956 German individuals, and 473 taxa from 5,959 Finland individuals. To ensure the authenticity and accuracy of the causal relationship between gut microbiota and longevity, the following quality control procedures were implemented to select IVs. Firstly, we selected IVs for each gut bacterial trait by using a loose cutoff of $P<1\times10^{-5}$. Secondly, the independent IVs with the lowest P-value for each trait ($r^2<0.001$ and distance = 10,000 kb) were retained to reduce the influence of correlations among SNPs. Thirdly, we calculated the F-statistic to evaluate the strength of the IVs. SNPs with F-statistics < 10 was disregarded to avoid weak IV bias. Fourthly, the screened SNPs were used as IVs to harmonize with summary statistic of longevity and the palindromic SNPs and fuzzy alleles were removed. At last, we removed SNPs with $P<1\times10^{-5}$ of outcome in harmonized data to avoid strong correlations between SNPs and outcome [14].

Statistical analysis

We primarily employed the inverse-variance weighted (IVW) method for our analysis. To assess the heterogeneity among SNPs, we conducted Cochran's *Q* test. If significant heterogeneity was observed ($P < 0.05$), we adopted the random-effects model; otherwise, the fixed-effects model was utilized [34]. The IVW method generated effect estimates for each SNP on gut microbiota and the likelihood of longevity, enabling us to compute the Wald estimates. Additionally, we performed sensitivity analyses to evaluate the robustness of our findings. The weighted median method was used to estimate the potential causal effects when IVs violated standard assumptions to provide a reliable estimate [35]. For the reliability of the final analysis results, the following screening criteria were used as filters for robust significant causality: (1) At least the IVW method suggested a significant causal relationship; (2) The direction of MR analysis results (beta value) was consistent among the three methods (IVW, MR-Egger, and weighted median); 3) We apply the maximum likelihood method or simple median method to replicate significant causal relationships, considering our reliance on the IVW method. MR-Egger method was used to detect directional pleiotropy, and intercept of $P > 0.05$ was deemed to be no horizontal pleiotropy [36]. Besides, the MR pleiotropy residual sum and outlier (MR-PRESSO) method was also applied to test for possible bias from horizontal pleiotropy and outlier variants removal [37]. Furthermore, the leave-one-out test was conducted to confirm that MR estimates were not driven by strong effect SNPs. The results were visually analyzed by forest plots and scatter plots. All MR analyses were performed in the R software (v4.2.3) using "Mendelian Randomization", "TwoSampleMR", and "MRPRESSO" packages. We considered suggestive evidence of a potential causal association when $P < 0.05$.

Results

Influence of the 403 gut bacterial taxa on longevity-related traits

We first investigate the causal relationships between the gut microbiome and longevity by performing a two-sample MR analysis using GWAS summary data of 403 gut bacterial taxa (196 taxa sourced from MiBioGen consortium, and 207 taxa from DMP) and longevity-associated traits. We observed suggestive evidence for many bacterial taxa to be associated with longevity, and these causal relationships were statistically significant with a *P*-value of less than 0.05, at least when employing the IVW method (Table S2**)**.

In terms of healthspan, our findings revealed that *Intestinimonas* (β = 0.033, $P = 0.049$), *Olsenella* (β = 0.044, $P = 0.002$), and *Turicibacter* (β = 0.045, $P = 0.016$) were positively correlated with healthspan, while *Anaerostipes* (β = − 0.051, $P = 0.034$), *Tyzzerella3* (β = − 0.030, $P = 0.026$), *Ruminiclostridium9* (β = − 0.059, $P = 0.012$), *Ruminococcus obeum* (β = − 0.045, $P = 0.030$), *Bacteroides xylanisolvens* (β = − 0.034, $P = 0.020$), *Bacteroides vulgatus* (β = -0.059, $P = 0.009$), and *Bacteroides eggerthii* (β = − 0.038, $P = 0.004$) were negatively correlated with healthspan. In the MR-Egger regression, there was no evidence of directional pleiotropic effects (intercept *p*-value > 0.05). There is a significant heterogeneity only for *Ruminococcus obeum* in the Cochran's *Q* test ($p = 0.031$).

For lifespan, *Defluviitaleaceae UCG-011*(β = 0.038, $P = 0.014$), *Erysipelotrichaceae UCG003* (β = 0.038, $P = 0.016$), *Senegalimassilia* (β = 0.052, $P = 0.002$), *Tyzzerella3* (β = 0.036, $P = 0.025$), *Odoribacter* (β = 0.031, $P = 0.030$), *Alistipes senegalensis* (β = 0.038, $P = 0.008$), *Bacteroides faecis* (β = 0.013, $P = 0.033$), *Holdemania unclassified* (β = 0.019, $P = 0.040$), and *Bilophila unclassified* (β = 0.030, $P = 0.045$) were positively associated with lifespan. In comparison, *Butyricimonas* (β = − 0.034, $P = 0.016$), *Lachnospira* (β = − 0.073, $P = 0.012$), *Lachnospiraceae UCG-001* (β = -0.037, $P = 0.014$), *Streptococcus salivarius* (Wald ratio, β = − 0.040, $P = 0.046$), and *Collinsella aerofaciens* (β = − 0.04, $P = 0.005$) were negatively associated with lifespan. Intercept of MR-Egger regression also showed no potential horizontal pleiotropy. There is a significant heterogeneity only for *Tyzzerella3* (Cochran's *Q* test, $p = 0.009$).

In relation to longevity, our findings revealed a positive correlation between *Bilophila wadsworthia* (β = 0.309, $P = 5 \times 10^{-4}$) and *Adlercreutzia equolifaciens* (β = 0.172, P = 0.022) with individuals who attained a lifespan exceeding the 90th percentile. *Lachnospiraceae bacterium 3_1_46FAA* was positively associated with longevity (> 90th percentile, β = 0.195, $P = 0.014$; > 99th percentile, β = 0.328, $P = 0.008$). On the contrary, *Blautia* (β = -0.246, $P = 0.001$), and *Escherichia coli* (β = -0.131, $P = 0.016$) were negatively correlated with longevity (> 90th percentile). *Akkermansia muciniphila* was negatively correlated with longevity (> 99th percentile, β = -0.219, $P = 0.030$). *Bacteroides massiliensis* was negatively associated with longevity (> 90th percentile, β = − 0.179, $P = 0.006$; > 99th percentile, β = − 0.216, $P = 0.038$). The forest plot and scatter plot about *B. massiliensis* were presented in Fig. 2 and Figure S1, while the outcomes of the leave-one-out analysis confirmed that the MR estimates were not driven by strong effect SNPs (Figure S2). In addition, *Haemophilus parainfluenzae* was also negatively associated with

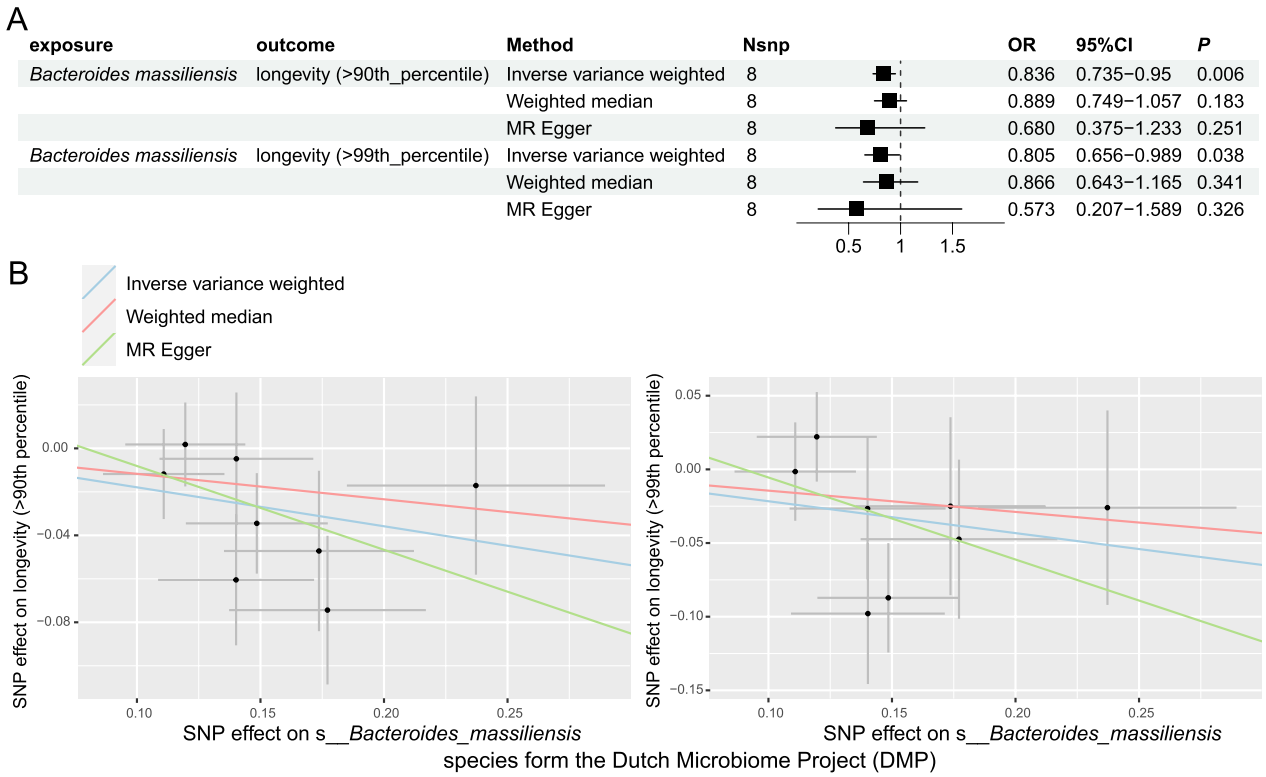


Fig. 2 The forest plot depicts the causal associations between *Bacteroides massiliensis* and traits associated with human longevity. The slope value equals the β-value calculated using the three methods (IVW, weighted median, and MR Egger), and it signifies the magnitude of the causal effect. A positive slope indicates that exposure is a contributory factor in promoting the outcome, whereas a negative slope suggests the opposite effect. Abbreviations: CI, confidence interval; OR, odds ratio; Nsnp, the number of single nucleotide polymorphisms (SNPs)

longevity (> 90th percentile, β = -0.154, $P = 0.011$; > 99th percentile, β = − 0.231, $P = 0.016$).

For parental longevity, genus *Anaerofilum* (β = 0.019, $P = 0.023$) was positively associated with father's age at death, while *Collinsella* (β = 0.035, $P = 0.035$), and *Eubacterium rectale* group (β = 0.046, $P = 0.005$) were positively associated with mother's age at death. *Desulfovibrio* (β = 0.031, $P = 0.036$), and *Eubacterium xylanophilum group* (β = 0.061, $P = 0.001$) were positively associated with combined parental age at death. *Slackia* (β = 0.033, $P = 0.002$), *Enterorhabdus* (β = 0.025, $P = 0.031$), and *Lachnospiraceae bacterium 5_1_63FAA* (β = 0.013, $P = 0.004$) increased parental longevity odds of father's attained age, while *Erysipelatoclostridium* (β = 0.017, $P = 0.044$), and *Eubacterium rectale* group (β = 0.041, $P = 0.038$) increased parental longevity odds of mother's attained age. Meanwhile, *Akkermansia muciniphila* was positively associated with mother's age at death (β = 0.021, $P = 0.033$), combined parental age at death (β = 0.025, $P = 0.012$), and both parents in top 10% (β = 0.012, $P = 0.047$). *Bacteroides fragilis* (β = 0.009, $P = 0.034$) and *Coprobacter fastidiosus* (β = 0.022, $P = 0.006$) were causally associated with mother's attained age. Moreover, *Eubacterium eligens* was positively associated with parental longevity (both parents in top 10%, β = 0.018, $P = 0.007$), while *Eubacterium rectale* was positively associated with combined parental age at death (β = 0.030, $P = 0.045$) in the IVW method. Regarding negative association, *Bacteroides* (β = − 0.060, $P = 0.006$), *Butyricicoccus* (β = -0.025, $P = 0.036$), *Flavonifractor* (β = − 0.041, $P = 0.036$), *Lachnospiraceae UCG008* (β = -0.018, $P = 0.020$), *Odoribacter* (β = − 0.045, $P = 0.043$), *Tyzzerella3* (β = − 0.015, $P = 0.022$), *Bacteroides dorei* (β = − 0.021, $P = 0.021$), and *Eubacterium biforme* (β = − 0.012, $P = 0.011$) were negatively linked to specific traits of parental longevity. Notably, *Oxalobacter* in the MiBioGen dataset was negatively associated with mother's age at death (β = − 0.019, $P = 0.038$), but *Oxalobacter* in the DMP dataset was positively associated with father's attained age (β = 0.011, $P = 0.048$).

Finally, frailty, which often accompanies aging, was analyzed as well. Genus *Bifidobacterium* (β = 0.042, $P = 0.013$), *Clostridium innocuum* group (β = 0.023, $P = 0.036$), *Eubacterium coprostanoligenes* group (β = 0.054, $P = 0.003$), *Flavonifractor* (β = 0.023, $P = 0.046$), and species *Ruminococcus torques* (β = 0.035, $P = 0.032$)

were positively associated with the frailty index (FI). The positive associations between the *Clostridium innocuum* group, the *Eubacterium coprostanoligenes* group, and frailty are consistent with a previous study[38].

Influence of the 903 gut bacterial taxa on longevity-related traits

The MR results of the significant associations between 903 gut bacterial taxa (430 taxa from 8956 German individuals, and 473 taxa from 5,959 Finland individuals in the FR02 cohort) and longevity-related phenotypes are summarized in Table S3.

For human healthspan, *Porphyromonadaceae* (β = 0.033, *P* = 0.001), *Atopobiaceae* (β = 0.065, *P* = 0.042), *Ruminococcaceae* (β = 0.021, *P* = 0.005), *Caloranaerobacter* (β = 0.140, *P* = 0.003), *Sutterella* (β = 0.035, *P* = 0.002), *Oscillibacter* prevalence (β = 0.019, *P* = 0.036), *Bifidobacterium breve* (β = 0.060, *P* = 0.018), *Lawsonibacter* sp002161175 (β = 0.124, *P* = 0.037), and *Monoglobus pectinilyticus* (β = 0.048, *P* = 0.017) were positively correlated with healthspan. On the contrary, *Alphaproteobacteria* (β = -0.038, *P* = 0.007), and *Coprococcus* (β = 0.124, *P* = 0.037) were negatively correlated with healthspan.

For lifespans, *Clostridium* XlVa (β = 0.074, *P* = 0.007), *Oscillibacter* abundance (β = 0.049, *P* = 0.005), *Ruminococcus*(β = 0.009, *P* = 0.020), and *Faecalibacterium prausnitzii E* (β = 0.063, *P* = 0.024) were positively correlated with lifespans, while *Roseburia* (β = − 0.042, *P* = 0.016), *Helicobacter* (β = − 0.121, *P* = 0.004), *Alistipes* (β = − 0.112, *P* = 0.038), *Photobacterium* (β = − 0.145, *P* = 0.024), *Desulfovibrio* (β = − 0.022, *P* = 0.001), *Parasutterella* prevalence (β = − 0.011, *P* = 0.049), *Oscillibacter* prevalence (β = − 0.016, *P* = 0.031), and *Lactococcus lactis* (β = -0.050, *P* = 0.036) were negatively correlated with lifespans. The significant causal relationship between *Oscillibacter* and longevity-related traits were summarized in Figure S3.

For longevity, *Parabacteroides* (β = 0.116, *P* = 0.035), *Gordonibacter* (β = 0.219, *P* = 0.030), *Sutterella* abundance (β = 0.103, *P* = 0.015), and *Alistipes shahii* (β = 0.166, *P* = 0.007) abundance in stool were linked to increased longevity (> 90th percentile). *Prevotella* sp900317685 (β = − 0.218, *P* = 0.013) and *Blautia* A sp900066355 (β = − 0.272, *P* = 0.026) were negatively correlated with longevity (> 90th percentile). *Prevotella* (β = − 0.065, *P* = 0.045), *Bacteroides* (β = − 0.068, *P* = 0.004), *Escherichia flexneri* (β = − 0.341, *P* = 0.002), and *Coprobacillus cateniformis* (β = − 0.376, *P* = 0.007), and *Parabacteroides johnsonii* (β = − 0.261, *P* = 0.047) were negatively correlated with longevity (> 99th percentile). Both the *Parasutterella* prevalence (β = − 0.085, *P* = 0.035) and *Parasutterella* abundance (β = − 0.078, *P* = 0.001) were negatively correlated with longevity (> 90th percentile).

Many of the 430 gut bacterial taxa from 8956 German individuals have been causally associated with parental longevity. *Alistipes* (β = 0.020, *P* = 0.009), and *Subdoligranulum* prevalence (β = 0.011, *P* = 0.025) were positively correlated with combined parental age at death. Alongside the previously mentioned results, more correlations between *Alistipes* and longevity were identified (Fig. 3A), especially the two *Alistipes* species of *A. senegalensis* and *A. shahii* (Fig. 3B). The results of the "leave-one-out" test showed that there was no abnormal IV in this analysis affecting the overall results (Figure S4). In addition, the *Desulfovibrio* abundance was negatively correlated with two parental longevity traits (both parents in top 10%, β = − 0.007, *P* = 0.038; combined parental age at death, β = − 0.015, *P* = 0.016), but was positively correlated with another two parental longevity traits (combined parental attained age, β = 0.016, $P = 2 \times 10^{-4}$; father's attained age, β = 0.013, *P* = 0.001). The *Sutterella* prevalence was negatively correlated with combined parental age at death (β = − 0.015, *P* = 0.045). *Faecalibacterium* abundance was negatively correlated with father's age at death (β = − 0.021, *P* = 0.008). More results were summarized in Table S3.

In addition, many of the 473 taxa from 5,959 Finland individuals have been causally associated with parental longevity. For example, *Leuconostoc mesenteroide* was positively associated with combined parental attained age (β = 0.019, *P* = 0.046). *Prevotella* sp000436915 was positively associated with combined parental attained age (β = 0.013, *P* = 0.048), but was negatively correlated with father's age at death (β = − 0.022, *P* = 0.005). *Bifidobacterium breve* was negatively correlated with both parents in top 10% (β = − 0.031, *P* = 0.002), but was positively correlated with combined parental attained age (β = 0.034, *P* = 0.010). *Bifidobacterium angulatum* was negatively correlated with mother's age at death (β = − 0.019, *P* = 0.043). *Dorea phocaeense* was negatively correlated with combined parental age at death (β = − 0.050, *P* = 0.025), but was positively associated with mother's attained age (β = 0.034, *P* = 0.018) and combined parental attained age (β = 0.035, *P* = 0.045). *Coprobacillus cateniformis* abundance was positively associated with father's age at death (β = 0.021, *P* = 0.036), but was negatively associated with mother's age at death (β = -0.030, *P* = 0.004). *Eubacterium callanderi* abundance was positively correlated with parental longevity (father's age at death) (β = 0.050, *P* = 0.020), but was negatively associated with father's attained age (β = − 0.042, *P* = 0.010). The abundance of *Lactococcus lactis* was positively correlated with father's attained age (β = 0.034, *P* = 0.025), and on the contrary, *Clostridium tertium* was negatively

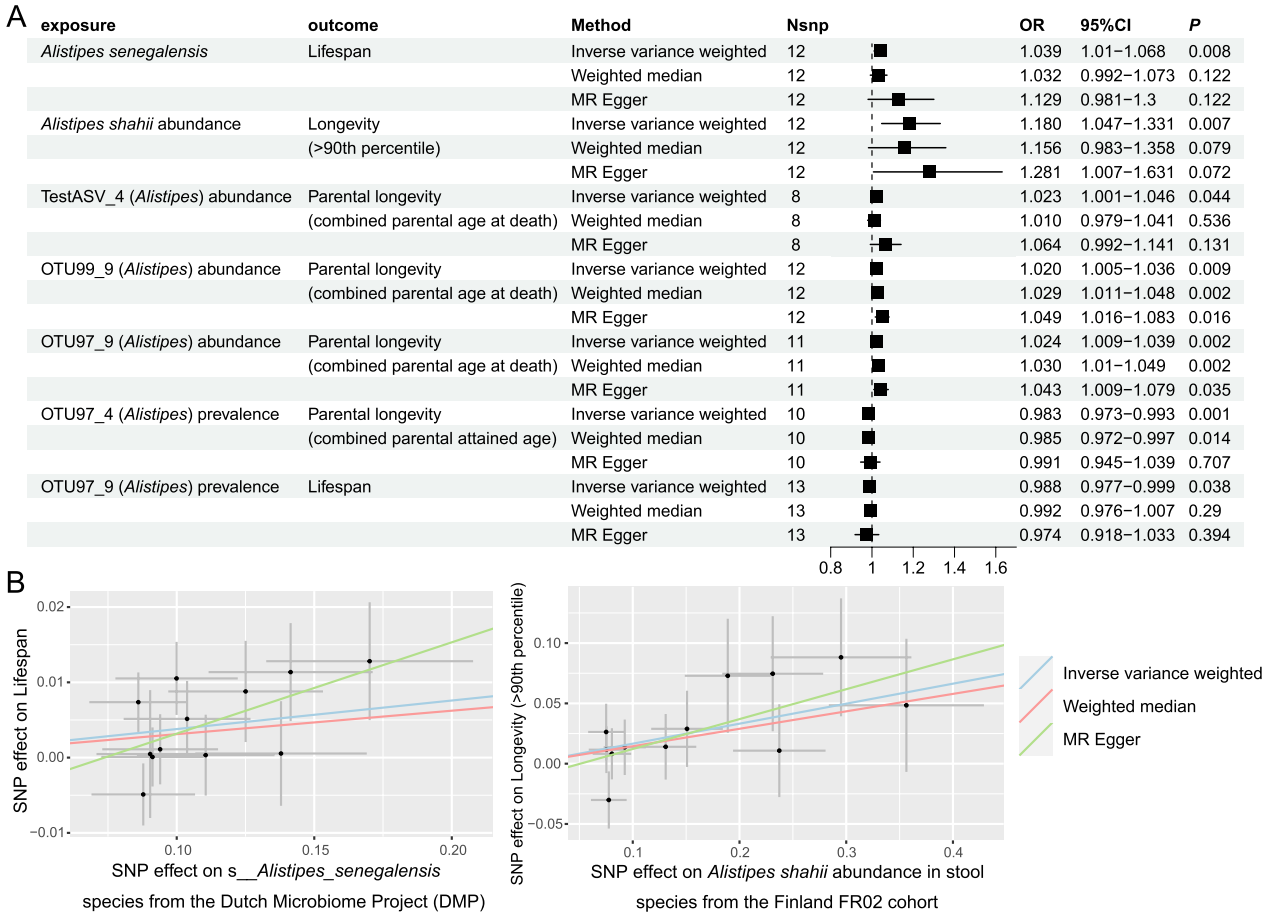


Fig. 3 MR results reveal the causal associations between *Alistipes* and traits associated with human longevity. **A** Forest plot depicting the associations between *Alistipes* and longevity-correlated traits. **B** Scatter plot depicting the associations between *Alistipes senegalensis* and lifespan, and between *Alistipes shahii* and longevity (> 90th percentile)

associated with it (β = − 0.051, *P* = 0.010). The abundance of *Enterococcus faecalis* in stool was positively correlated with mother's attained age (β = 0.027, *P* = 0.045), while *Blautia A* sp900066355 was negatively linked to it (β = -0.036, *P* = 0.007). *Enorma massiliensis* was positively correlated with mother's attained age (β = 0.027, *P* = 0.013) but was negatively correlated with father's attained age (β = − 0.025, *P* = 0.022).

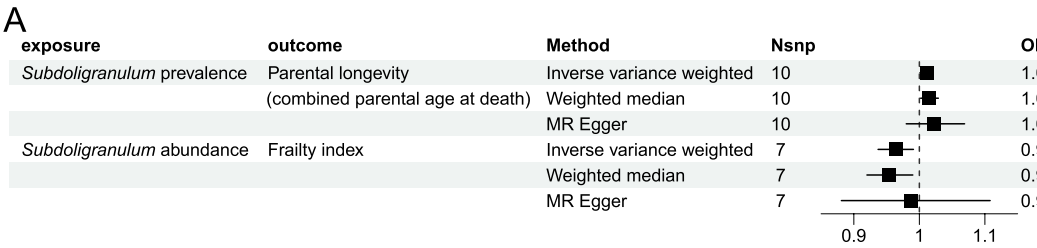
The MR results of the association between 903 gut bacterial taxa and frailty indicated that *Sutterella* prevalence (OTU99_116, β = 0.009, *P* = 0.009; TestASV_22, β = 0.014, *P* = 0.002) was positively correlated with FI, while the *Sutterella* abundance (β = − 0.040, *P* = 0.004) and *Parasutterella* abundance (β = -0.008, *P* = 0.021) were negatively correlated with FI. The abundance of *Faecalibacterium* (β = 0.033, *P* = 0.015), and *Anaeromassilibacillus* sp001305115 (β = 0.047, *P* = 0.003) were positively correlated with FI. *Bacteroides* sp002160055 (β = − 0.036, *P* = 0.017), *Bacteroides stercoris* (β = − 0.025, *P* = 0.032), *Lawsonibacter* sp002161175 (β = − 0.081, *P* = 0.037), *Morganella* (β = − 0.038, *P* = 0.042), and *Subdoligranulum* abundance (β = − 0.037, *P* = 0.009) in stool were negatively correlated with FI, at least in the IVW method. To sum up, *Subdoligranulum* was causally associated with multiple longevity-correlated traits (Fig. 4), and no abnormal IV in this analysis affecting the overall results (Figure S5). Moreover, all the notable associations between *Parasutterella* and longevity-related traits indicate *Parasutterella* has a negative impact on lifespan, longevity, and frailty (Figure S6).

Influence of the 205 gut functional pathways on longevity-related traits

The gut microbial taxa potentially regulate longevity through their associated metabolic pathways, prompting us to also analyze the influence of gut functional pathways on longevity-related traits. Only the gut microbiome from 7,738 participants of the DMP encompassed 205 functional pathways [32], whereas the other three GWAS datasets of gut microbiota exclusively comprised

A

exposure	outcome	Method	Nsnp	OR	95%CI	P
Subdoligranulum prevalence	Parental longevity	Inverse variance weighted	10	1.011	1.001−1.021	0.025
	(combined parental age at death)	Weighted median	10	1.015	1.002−1.029	0.027
		MR Egger	10	1.023	0.98−1.069	0.329
Subdoligranulum abundance	Frailty index	Inverse variance weighted	7	0.964	0.937−0.991	0.009
		Weighted median	7	0.954	0.92−0.99	0.012
		MR Egger	7	0.988	0.881−1.108	0.84



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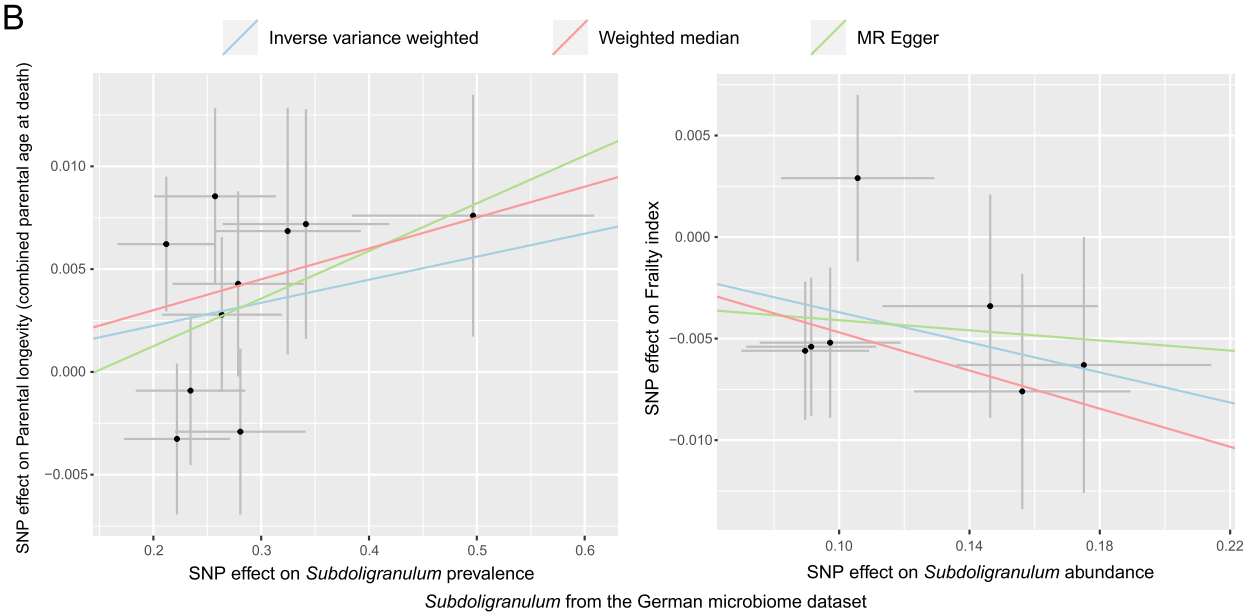

Fig. 4 The causal associations between *Subdoligranulum* and traits associated with human longevity. The forest plot **A** and scatter plot **B** depicting the association between *Subdoligranulum* prevalence and Parental longevity (combined parental age at death), and between *Subdoligranulum* abundance and frailty index, respectively

microbial taxa. A total of 65 pathways have been discovered to exhibit significant and robust associations with traits linked to longevity (Table S4). There was no evidence of directional pleiotropic effects (intercept p-value > 0.05), and no significant heterogeneity (Cochran's Q test, p-value > 0.05).

For human healthspan, microbial pathways of PPGPP-MET-PWY: ppGpp biosynthesis (β = 0.037, p = 0.028), and PWY-6507: 4-deoxy-L-threo-hex-4-enopyranuronate degradation (β = 0.017, p = 0.044) showed a positive association, in the IVW method. In contrast, pathways such as PWY-6284: superpathway of unsaturated fatty acids biosynthesis (*E. coli*) (β = −0.049, p = 0.005), HOMOSER-METSYN-PWY: L-methionine biosynthesis I (β = −0.051, p = 0.011), DENOVOPURINE2-PWY: superpathway of purine nucleotides de novo biosynthesis II (β = −0.042, p = 0.030), PWY-6163: chorismate biosynthesis from 3-dehydroquinate (β = −0.043, p = 0.035), and PWY-5913: TCA cycle VI obligate autotrophs (β = −0.047, p = 0.004), were negatively correlated with healthspan.

For lifespan, pathways of NONOXIPENT-PWY: pentose phosphate pathway (non-oxidative branch) (β = 0.055, P = 0.002), PWY-6630: superpathway of L-tyrosine biosynthesis (β = 0.023, P = 0.008), PWY-7196: superpathway of pyrimidine ribonucleosides salvage (β = 0.047, P = 0.011), PWY-7209: superpathway of pyrimidine ribonucleosides degradation (β = 0.022, P = 0.032), and PWY0-162: superpathway of pyrimidine ribonucleotides de novo biosynthesis (β = 0.030, P = 0.033) were positively associated. In contrast, UBISYN-PWY: superpathway of ubiquinol 8 biosynthesis (prokaryotic) (β = -0.040, P = 0.001), KDO-NAGLIPASYN-PWY: superpathway of $(Kdo)_2$-lipid A biosynthesis (β = − 0.024, P = 0.006), PWY-7211: superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (β = -0.032, P = 0.017), PWY_REDCITCYC: TCA cycle VIII (helicobacter) (β = -0.030, P = 0.007), PWY-5918: superpathway of heme biosynthesis from glutamate (β = -0.028, P = 0.022), PWY0-1338: polymyxin resistance (β = − 0.020, P = 0.023), and ENTBACSYN-PWY: enterobactin biosynthesis

(β = − 0.039, P = 0.028) demonstrated negative associations with lifespan.

Regarding longevity, the abundances of PWY-6731: starch degradation III was positively associated with age > 90th percentile (β = 0.084, P = 0.020), while PWY-5941: glycogen degradation II (eukaryotic) was positively associated with age > 99th percentile (β = 0.262, P = 0.030). Moreover, THRESYN-PWY: superpathway of L-threonine biosynthesis (β = − 0.338, P = 0.017), and PWY0-1261: anhydromuropeptides recycling (β = − 0.346, P = 0.020) were negatively correlated with longevity (age > 99th percentile).

Regarding parental longevity, 46 associations have been identified. For instance, PWY-7197: pyrimidine deoxyribonucleotide phosphorylation was positively associated with father's attained age in the IVW method (β = 0.019, P = 0.045). PWY-7254: TCA cycle VII (acetate producers) was positively associated with mother's age at death (β = 0.016, P = 0.046). PWY-7456: mannan degradation was positively associated with father's attained age (β = 0.031, P = 0.001). PWY-6897: thiamin salvage II was positively associated with both parents in top 10% (β = 0.023, P = 0.007). PWY-5088: L-glutamate degradation VIII to propanoate were positively associated with father's attained age (β = 0.015, P = 0.009). GALACTARDEG-PWY: D-galactarate degradation I was positively associated with mother's attained age (β = 0.014, P = 0.021), and combined parental attained age (β = 0.014, P = 0.045). On the contrary, PWY-7323: superpathway of GDP-mannose derived O-antigen building blocks biosynthesis (β = -0.026, P = 0.014) was negatively associated with parental longevity (father's age at death). PWY-5838: superpathway of menaquinol-8 biosynthesis I (β = − 0.022, P = 0.043), and HOMOSER-METSYN-PWY: L-methionine biosynthesis I (β = − 0.029, P = 0.043) were negatively correlated with mother's age at death. PWY-5667: CDP-diacylglycerol biosynthesis I was negatively correlated with father's attained age (β = − 0.019, P = 0.012). PWY0-1415: superpathway of heme biosynthesis from uroporphyrinogen III (β = − 0.016, P = 0.012), DAPLYSINESYN-PWY: L-lysine biosynthesis I (β = − 0.021, P = 0.017), and ANAEROFRUCAT-PWY: homolactic fermentation (β = − 0.037, P = 0.029) were negatively correlated with combined parental attained age. METHGLYUT-PWY: superpathway of methylglyoxal degradation (β = − 0.008, P = 0.034), and ORNDEG-PWY: superpathway of ornithine degradation (β = − 0.010, P = 0.027) were negatively correlated with both parents in top 10%. Notably, COA-PWY (coenzyme A biosynthesis I) exhibited a positive association with father's age at death (β = 0.035, P = 0.013) and combined parental age at death (β = 0.038, P = 0.023), but exhibited a negative association with combined parental attained age (β = − 0.030, P = 0.002), as illustrated in Fig. 5A. The pathway of P162-PWY: L-glutamate degradation V via hydroxyglutarate was negatively correlated with mother's age at death (β = − 0.028, P = 0.012) and both parents in top 10% (β = -0.020, P = 0.014), but was positively correlated with mother's attained age (β = 0.018, P = 0.044) and combined parental attained age (β = 0.019, P = 0.034). PWY-7013: L-1,2-propanediol degradation exhibited a negative correlation with father's age at death (β = -0.012, P = 0.034) and combined parental age at death (β = − 0.016, P = 0.042), but exhibited a positive correlation with father's attained age (β = 0.008, P = 0.043) and combined parental attained age (β = 0.009, P = 0.042).

In terms of frailty-correlated pathways, we found PWY-7456: mannan degradation (β = − 0.056, P = 0.008), TRNA-CHARGING-PWY: tRNA charging (β = − 0.032, P = 0.027), POLYAMSYN-PWY: superpathway of polyamine biosynthesis I (β = − 0.062, P = 0.003), and PWY-5101: L-isoleucine biosynthesis II (β = -0.032, P = 0.019) were negatively correlated with frailty index. In comparison, PWY-5920: superpathway of heme biosynthesis from glycine (β = 0.013, P = 0.044), PWY-6630: superpathway of L-tyrosine biosynthesis (β = 0.021, P = 0.049), GLUCONEO-PWY: gluconeogenesis I (β = 0.054, P = 0.009), and GLUCOSE1PMETAB-PWY: glucose and glucose 1-phosphate degradation (β = 0.036, P = 0.026) were positively associated with frailty index.

There are several multiple associations between specific one pathway and longevity traits among different datasets (Table S5). For example, the PWY-5100 (pyruvate fermentation to acetate and lactate II) pathway was positively associated with both lifespan (β = 0.028, P = 0.039) and parental longevity of combined parental age at death (β = 0.032, P = 0.009), but was negatively correlated with father's attained age (β = − 0.022, P = 0.006), which was depicted in Fig. 5B. The NONOXIPEN-PWY: pentose phosphate pathway (non-oxidative branch) exhibited a positive association with lifespan (β = 0.055, P = 0.002), parental longevity of both parents in top 10% (β = 0.026, P = 0.009), and combined parental age at death (β = 0.051, P = 0.006), but exhibited a negative association with mother's attained age (β = − 0.026, P = 0.017), father's attained age (β = − 0.024, P = 0.032), and combined parental attained age (β = -0.036, P = 0.001) (Fig. 6A). The PWY-7209: superpathway of pyrimidine ribonucleosides degradation exhibited a positive association with lifespan (β = 0.022, P = 0.032), but exhibited a opposite association with parental longevity of mother's attained age (β = − 0.016, P = 0.040) and combined parental attained age (β = -0.013, P = 0.046). Moreover, the pathway of REDCITCYC: TCA cycle VIII (helicobacter) was consistently negatively associated with lifespan, and three traits of parental longevity (both parents in top

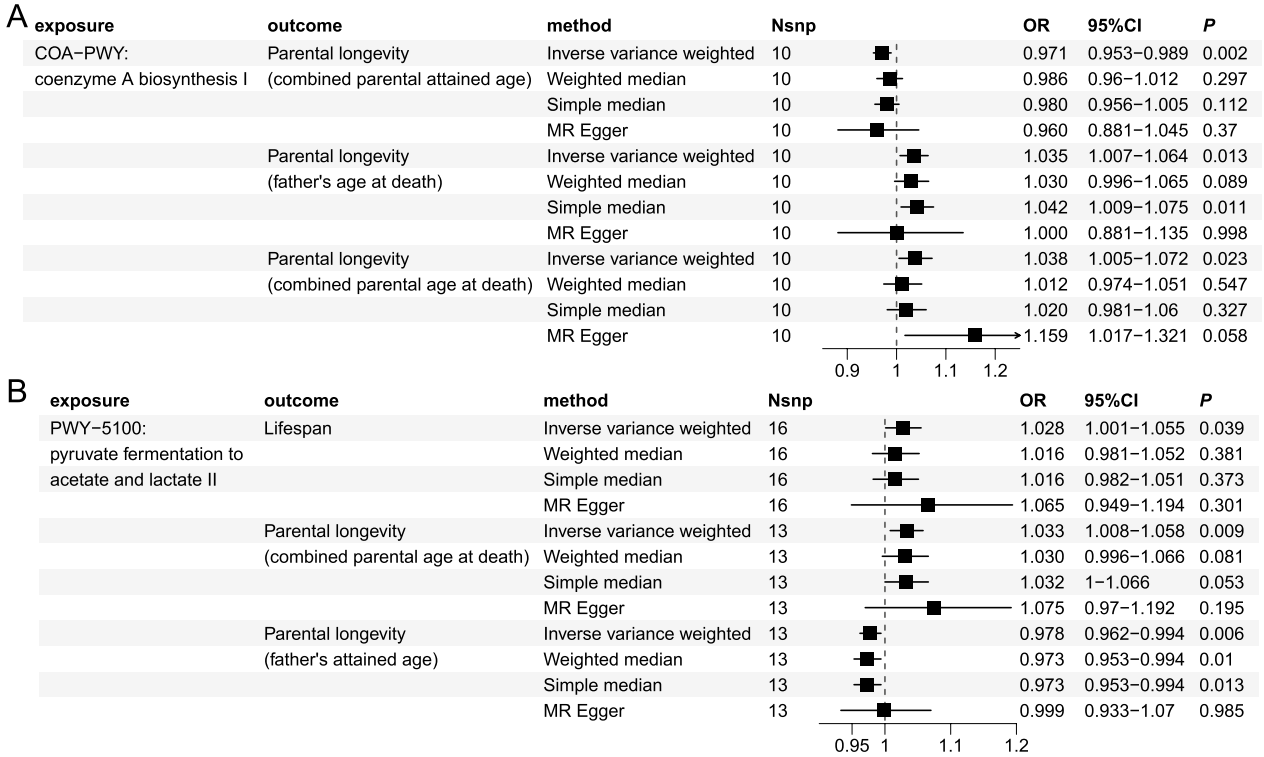

Fig. 5 The forest plots reveal the impact of two microbial pathways, COA-PWY and PWY-5100, on traits associated with longevity. **A** The notable associations between COA-PWY (coenzyme A biosynthesis I) pathway and parental longevity. **B** The notable associations between PWY-5100 (pyruvate fermentation to acetate and lactate II) and traits associated with lifespan and parental longevity. Four MR methods including IVW, weighted median, simple median, and MR Egger were used. The causal relationships were presented using OR (odds ratio) and 95% CI (confidence interval)

10%, mother's age at death, and combined parental age at death) (Fig. 6B). The of HOMOSER-METSYN-PWY (L-methionine biosynthesis I) was consistently negatively associated with lifespan, and parental longevity of mother's age at death. The PWY-6507 (4-deoxy-L-threo-hex-4-enopyranuronate degradation) pathway was consistently positively associated with lifespan and parental longevity (both parents in top 10%). In addition, the PWY-7456 (mannan degradation) pathway was positively associated with parental longevity of father's attained age, and negatively associated with FI.

Discussion

In this study, we employed a two-sample MR based on GWAS summary data to extensively investigate the potential causal effects between gut microbiota and longevity. To the best of our knowledge, this study used the most comprehensive datasets currently available, including five publicly available GWAS datasets of longevity-related traits and four GWAS datasets of gut microbiota.

Previous research has established a significant correlation between *Collinsella* and parental longevity [23]. We found *Collinsella* was positively associated with parental longevity of mother's age at death, but *Collinsella aerofaciens* was negatively associated with lifespan. Moreover, Liu et al. [24] assessed the causal relationships between human microbiome and longevity by MR analyses in Chinese populations based on GWAS summary statistics of the gut and oral microbiome from the 4D-SZ cohort [39] and longevity from the CLHLS cohort [40], and found genus *Oxalobacter* and species *Lactobacillus amylovorus* were positively associated with longevity. Our results indicate that *Oxalobacter* exhibits a positive correlation with one aspect of parental longevity, specifically the attained age of the father, whereas it demonstrates a negative association with three other characteristics of parental longevity, namely the mother's age at death, the father's age at death, and the combined age at death of both parents. *Oxalobacter formigenes*, one typical species of *Oxalobacter* genus, is a key oxalate-degrading bacterium in the mammalian intestinal tract and can reduce the risk of calcium oxalate kidney stone disease [41, 42]. Therefore, *Oxalobacter* spp may protect humans from kidney stone disease and promote their overall health.

We also observed a negative association between *Bacteroides massiliensis* and longevity (age > 90th percentile,

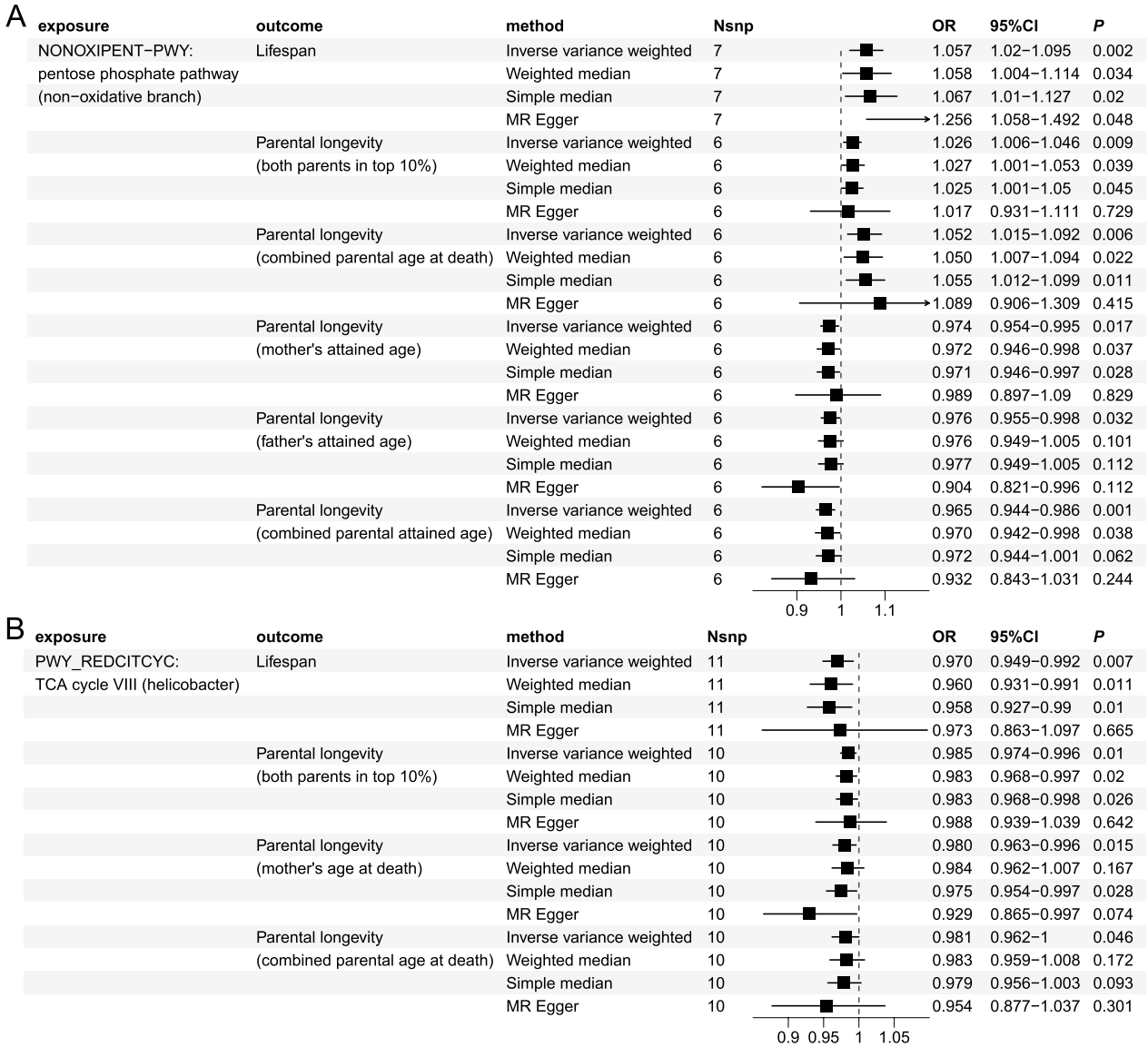

Fig. 6 The forest plots reveal the impact of two significant microbial pathways, NONOXIPEN-PWY and REDCITCYC, on multiple traits associated with longevity. **A** The notable associations between NONOXIPEN-PWY (pentose phosphate pathway, non-oxidative branch) pathway and traits associated with longevity. **B** The notable associations between REDCITCYC: TCA cycle VIII (helicobacter) pathway and traits associated with longevity. Four MR methods including IVW, weighted median, simple median, and MR Egger were used. The causal relationships were presented using OR (odds ratio) and 95% CI (confidence interval)

and > 99th percentile), indicating *Bacteroides massiliensis* may decrease the likelihood of achieving longevity. A higher relative abundance of *B. massiliensis* was observed in prostate cancer cases compared to benign controls [43]. A potential mechanism underlying this association may be the possession of glucuronidase genes by *B. massiliensis*, which, through its glucuronidase deconjugation activity, leads to elevated levels of free estrogens in the bloodstream. These increased estrogens subsequently create apurinic sites within DNA, causing mutations that may stimulate the onset of oncogenesis [44]. Moreover, the *Oscillibacter* abundance was positively correlated with lifespan and two traits of parental longevity, but was negatively associated with another two traits of parental longevity (mother's attained age, and combined parental attained age). The *Alistipes* abundance was positively correlated with combined parental age at death. More specific, *Alistipes senegalensis* was positively associated with lifespan, while *Alistipes shahii* abundance in stool was linked to increased longevity

(age > 90th percentile). Interestingly, an increased relative abundance of fecal *Oscillibacter* and *Alistipes* has been causally linked to a decreased triglyceride concentration [39]. Species from the *Oscillibacter* genus involve cholesterol metabolism and show potential benefits for lipid homeostasis and cardiovascular health [45]. Furthermore, *Alistipes* may exhibit protective effects against liver cirrhosis and cardiovascular diseases [46]. Therefore, the positive effects of *Oscillibacter* and *Alistipes* spp on longevity probably be mediated by lipid homeostasis and cardiovascular health in human subjects. Additionally, the *Subdoligranulum* prevalence was positively correlated with parental longevity (combined parental age at death), and the *Subdoligranulum* abundance was negatively associated with frailty. It is noteworthy that the genera *Subdoligranulum* correlated with slower biological aging [47], potentially attributed to the production of anti-inflammatory short-chain fatty acids. *Subdoligranulum* was strongly associated with *A. muciniphila* in an overweight/obese population; however, the supplementation with a *Subdoligranulum variabile* strain in obese and diabetic mice did not yield beneficial effects [48]. There is a critical need for more extensive animal studies for *Subdoligranulum* spp, such as in aging-related animal models.

The casual association of some taxa on longevity are inconsistent. For instance, *Akkermansia muciniphila* was negatively correlated with longevity (age > 99th percentile), but was positively associated with three traits of parental longevity (mother's age at death, combined parental age at death, and both parents in top 10%). In addition, *Desulfovibrio* in the MiBioGen dataset was positively associated with parental longevity of combined parental age at death. *Desulfovibrio* in the German dataset was negatively correlated with lifespans and two parental longevity traits (both parents in top 10%, and combined parental age at death), while was positively associated with father's attained age and combined parental attained age. *D. piger*, a representative species of the *Desulfovibrio* genus, is identified as one of the gut microbes associated with human aging [49]. *Flavonifractor* was positively correlated with longevity (age > 99th percentile) and frailty, and was negatively correlated with parental longevity. One species of *Flavonifractor* genus, *Flavonifractor plautii*, has been found to protect against elevated arterial stiffness [50], but correlated with colorectal cancer [51, 52]. Consistently, *Parasutterella* exhibited a negative correlation with both lifespan and longevity (age > 90th percentile). *Parasutterella* was positively associated with BMI and type 2 diabetes [53], suggesting it may potentially affect metabolic diseases. Moreover, *Blautia A* sp900066355 was negatively correlated with longevity (age > 90th percentile) and parental longevity of mother's attained age. The mechanisms by which *Desulfovibrio* spp, *Flavonifractor* spp, *Parasutterella* spp, and *Blautia A* sp900066355 may contribute to disease pathology or healthy aging remains to be investigated.

As for microbial pathways, the abundance of PWY-6731(starch degradation III) was positively associated with longevity of age > 90th percentile. A previous GWAS study indicated that the starch, sucrose and xenobiotic metabolism pathway was highly associated with human longevity in Han Chinese [40]. Species such as *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Roseburia inulinivorans* involve in the starch degradation and exert health-regulating effects by producing short-chain fatty acids (SCFAs) [54]. Thus, the starch degradation pathway may have a profound impact on longevity. COA-PWY (coenzyme A biosynthesis I), PWY-5100 (pyruvate fermentation to acetate and lactate II), and NONOX-IPEN-PWY (pentose phosphate pathway, non-oxidative branch) exhibited a positive correlation with two or more longevity traits. Previous studies have revealed that acetyl-coenzyme A as a phylogenetically conserved inhibitor of age-associated autophagy and prolongs lifespan [55, 56]. In addition, the *Sis2* gene regulated yeast lifespan through the coenzyme A biosynthesis pathway [57], implying a role for this pathway in longevity regulation. Lactate promotes oxidative stress resistance through hormetic ROS signaling [58], while acetate metabolism play a role in the regulation of aging and longevity [59]. The pentose phosphate pathway is crucial for cellular redox balance, and helps to maintain mitochondrial reactive oxygen species homeostasis and to extend lifespan in *C. elegans* [60]. Researchers also discovered that long-lived flies exhibit a delayed age-related decline in protein turnover rates and elevated carbon flux into the pentose phosphate pathway [61]. These findings support a causal link between pentose phosphate pathway and lifespan extension. Furthermore, the TCA cycle VIII (helicobacter) pathway was consistently negatively associated with lifespan and three traits of parental longevity. The *Helicobacter*-specific tricarboxylic acid cycle (TCA cycle VIII) was associated with gastric carcinogenesis [62], indicating a potential harmful mechanism that contributes to the suppression of longevity. These pathways could serve as targets for future therapeutic approaches for aging-related disorders.

These findings have profound implications for our understanding of the causal relationships between the gut microbiome and human longevity. By identifying specific gut microbial taxa and their functional pathways associated with longevity, we gain a more precise picture of which microbial features may influence human lifespan. They not only enhance our understanding of

longevity mechanisms but also pave the way for microbiome-targeted interventions to promote healthy aging. For instance, if we increase our intake of dietary fiber to enhance the abundance of starch degradation III pathway or appropriately use medications to eliminate *Helicobacter pylori* when *H. pylori* infection has been diagnosed, it is likely to improve our health.

Conclusions

This study reveals numerous causal associations between gut microbiota and longevity. *Bacteroides massiliensis* and *Parasutterella* were negatively associated with longevity, while *Alistipes* and *Subdoligranulum* exhibited a positive correlation with longevity. Microbial pathways of coenzyme A biosynthesis I, pyruvate fermentation to acetate and lactate II, pentose phosphate pathway, and TCA cycle VIII (helicobacter) were notably associated with longevity in a potentially protective or detrimental manner. More extensive population-based observational studies and longitudinal studies, as well as animal experiments, are needed to elucidate these causal associations and their underlying mechanisms.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05823-2.

Supplementary material 1: Figure S1. The forest plot depicts the MR effect size for species *Bacteroides massiliensis* on longevity (age >90th percentile, and age >99th percentile) and frailty.

Supplementary material 2: Figure S2. The MR leave-one-out sensitivity analysis for *Bacteroides massiliensis* on longevity (>90th percentile, and >99th percentile).

Supplementary material 3: Figure S3. The forest plot depicts the causal associations between *Oscillibacter* prevalence, as well as *Oscillibacter* abundance, and traits associated with human longevity.

Supplementary material 4: Figure S4. The MR leave-one-out sensitivity analysis for *Alistipes senegalensis* on lifespan, and for *Alistipes shahii* on longevity (>90th percentile).

Supplementary material 5: Figure S5. The MR leave-one-out sensitivity analysis for *Subdoligranulum* prevalence on Parental longevity (combined parental age at death), and for *Subdoligranulum* abundance on frailty index.

Supplementary material 6: Figure S6. The forest plot depicts the causal associations between *Parasutterella* and longevity-related traits, including frailty. All these associations were negative.

Supplementary material 7: Table S1. Details of the genome-wide association studies and datasets used in our Mendelian Randomization (MR) study.

Supplementary material 8: Table S2. The significant influence of 403 gut bacterial taxa from MiBioGen and DMP datasets on longevity-related phenotypes.

Supplementary material 9: Table S3. The significant influence of 903 gut bacterial taxa from German and Finland individuals on longevity-related phenotypes.

Supplementary material 10: Table S4. The significant influence of the 205 microbial pathways from DMP dataset on specific longevity-related phenotypes.

Supplementary material 11: Table S5. The MR results reveal the notable associations between specific one microbial pathway and multiple longevity-related traits among different datasets.

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Author contributions
Sheng Liu contributed to study design. Shu Chen and Wei Chen collected, and analyzed the data. Xudong Wang contributed to the visualization. Shu Chen drafted the manuscript. Sheng Liu revised the manuscript. All authors have read and approved the final manuscript.

Data availability
The datasets analyzed in the current study can be downloaded from the websites that were listed in supplementary Table 1.

Declarations

Ethics approval and concern to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
No conflicts of interest to declare.

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