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The causal role of gut microbiota in development of osteoarthritis

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SUMMARY

Objective: There is considerable evidence for relationship between gut microbiota and osteoarthritis (OA), but no studies have investigated their causal relationship.
Method: This study utilized large-scale genome-wide association studies (GWAS) summary statistics to evaluate the causal association between gut microbiota and OA risk. Specifically, two-sample Mendelian randomization (MR) approach was used to identify the causal microbial taxa for OA. Comprehensively sensitive analyses were performed to validate the robustness of results and novel multivariable MR analyses were further conducted to ensure the independence of causal association. Reverse-direction MR analyses were performed to rule out the possibility of reverse associations. Finally, enrichment analyses were used to investigate the biofunction.
Results: After correction, three microbial taxa were identified to be causally associated with diverse joint OA ($P_{FDR} < 0.100$), namely *Methanobacteriaceae* family for knee OA ($P_{FDR} = 0.043$) and any OA ($P_{FDR} = 0.028$), *Desulfovibrionales* order for knee OA ($P_{FDR} = 0.045$) and *Ruminiclostridium5* genus for knee OA ($P_{FDR} = 0.063$). In addition, we also identified five suggestive microbial taxa that were significant with three different methods under the nominal significance ($P < 0.05$). Sensitive analysis excluded the influence of heterogeneity and horizontal pleiotropy and multivariable MR analysis ruled out the possibility of horizontal pleiotropy of BMI. GO enrichment analysis illustrates the protective mechanism of the identified taxa against OA.
Conclusions: This study found that several microbial taxa were causally associated with diverse joint OA. The results enhanced our understanding of gut microbiota in the pathology of OA.

Introduction

Osteoarthritis (OA) is one of leading causes of pain and disability in the elderly worldwide and can add significantly to socio-economic costs[1,2]. OA is increasingly common than in the past due to ageing and the increase in obesity[3]. Although OA is becoming relatively prevalent, most sufferers are not being managed with timely and appropriate treatment[4]. OA is an incurable disease, and most treatments are aimed at relieving symptoms, which reinforces the importance of early diagnosis of OA. Early detection of OA is a challenging task as the link between pain and structural degeneration is not strong. Recently, an increasing number of genetic loci associated with OA have been identified through genome-wide association studies (GWASs)[5,6]. For instance, a large-scale genome-wide association study (GWAS) meta-analysis identified 52 novel OA-related signals, and mapped six signals precisely to a single variant[7]. These findings bring new insights into the etiology of OA from a genetic perspective.

The human gastrointestinal tract is inhabited by trillions of symbiotic bacteria that have a reciprocal symbiotic relationship with the human body and which help to sustain our health[8]. With the continued development of high-throughput sequencing technologies and platforms, there is growing evidence that the gut biome plays an important role in the skeletal metabolic program. Gut microbes are involved in a variety of histological functions, including metabolic, immune and neurological, maintaining metabolic stability, development of the immune system, resistance to infection and production of certain neurotransmitters[9]. As the balance of the gut bacteria is interrupted by various factors, individuals can suffer from obesity, diabetes, metabolic diseases and

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even cancer[10]. A growing number of studies have demonstrated that gut microbes are intimately involved in the metabolism of bone tissue, but the mechanisms underlying this have not been completely explained. With the continuous development of modern biomolecular technology and macro-data, human recognition of gut microbes is improving, which makes it feasible to detect the early pathogenesis of OA[11].

There is increasing interest in the relationship between gut microbiota and OA. The increasing trend of OA is not just due to increased life expectancy, but also to unhealthy lifestyles, such as reduced physical activity and an unhealthy diet, which can lead to obesity and chronic inflammation[12]. Adverse change in gut microbiota may lead to metabolic syndrome and inflammation, both of which are also important components in inducing the development and progression of OA[13]. It is widely accepted that gut microbiota could contribute to complicated interactions between mechanical, cellular and biochemical factors, which is crucial in OA pathogenesis and increased susceptibility to OA[14]. Therefore, alterations in the microbiological composition of the gut may be a bridge between these adverse factors and the development of OA. New evidence has indicated that gut microbiota promotes systemic inflammation through activation of the innate immune response, which may represent a link between metabolism and the mechanisms of OA development[15]. A recent study conducted by Lorenzo *et al.* indicated substantial changes in the gut and oral microbiota of sufferers of rheumatoid arthritis and OA[16]. Solovieva *et al.* discovered that vitamin D receptor (VDR) gene polymorphisms play a pathogenetic role in symmetrical hand OA in a Finnish group of humans[17]. However, previous epidemiology studies could not rule out the influence of unavoidable confounding during the long-term development of OA.

Recently, a large-scale association study gave the evidence that microbiome loci are enriched in metabolic, nutritional and environmental domains, identifying causal roles of gut microbiota in ulcerative colitis and rheumatoid arthritis[18]. This provides an unprecedented opportunity to further explore the causal gut microbiota in osteoarthritis. A two-sample Mendelian Randomization (MR) study assessed the association between gut microbiome as a whole and OA, but there were no encouraging findings[19]. However, microbiome composition is extremely complex and diverse, and different gut microbiota have distinct effects on human health. Therefore, it is inappropriate to merge all gut microbiota to an indicator. To the best of our knowledge, no studies have extensively assessed the causal association between gut microbiota and OA. This study aims to explore the contribution of gut microbial imbalance in the progression of OA by examining the interaction between the gut microbiota and OA through MR, and to further provide new insights into the early detection and diagnosis of OA. Overview of our research is presented in Fig. 1.

Materials and methods

GWAS summary statistics

The data sets used in our analysis were all public available (Table I): GWAS summary statistics for gut microbiota served as exposure that could be downloaded from MiBioGen consortium (http://www.mibiogen.org/)[18]. Gut microbiota in this study were divided into 211 taxa (131 genera, 35 families, 20 orders, 16 classes and 9 phyla) and a total of 5,717,754 SNPs genotyped by 16S fecal microbiome were analyzed on 18,340 individuals (24 cohorts). For genotype imputation, the HRC 1.0 or 1.1 reference panel was used. Then association analysis was performed by spearman correlation with covariates as age, sex, technical covariates, and genetic PCs. GWAS summary statistics for OA (knee, hip, Knee represents OA (knee) and/or hip, and any OA) were generated by a meta-analysis of the UK Biobank (N = ~500,000) and arcOGEN (N = ~17,000) and were released to the public[7]. A total of 37,106,466 SNPs were evaluated, and the association analyses were performed with adjustment for sex, age, chip and genetic PCs. As exposure and outcome data were obtained from different consortiums, the degree of sample overlap between them is low[20]. Finally, the useful information (e.g., effect size, standard error, effect allele and P value) for each SNP was remained for further analysis.

Selection of instrumental variables

Following[18,21], a loose cutoff of $P < 1E\text{-}5$ was used to choose significant SNPs for each gut microbial taxa. The clump procedure in PLINK software was used to rule out the dependent instrumental variables with $r^2 < 0.001$ based on the European-based 1,000 Genome Projects reference panel[22]. If the IVs were missing in outcome data sets, then proxies were added with the $r^2 > 0.8$. Then a total of 4–25 independent IVs were remained for further analysis. To quantify the strength of IVs, we calculated the proportion of variation explained (PVE) and the F statistic for each IV of microbial taxa. Detailed information of selected SNPs was summarized in Table S1. Additionally, in reverse-direction MR analysis, we selected IVs for each OA phenotypes by using a much stricter threshold, where the significant threshold was set to be 5E-8 and r^2 was set to be 0.01.

Estimation of causal effect and sensitivity analysis

Three methods were mainly used to estimate the causal effects: fixed/random-effect inverse variance weighting (IVW) method[23], weighted median method[24], and MR-Egger regression[25]. Different methods could provide valid evidence under different conditions. Cochran Q test was first used to test the heterogeneity among SNPs included in each analysis, and the random-effect IVW will be utilized if heterogeneity exists. Then comprehensive sensitivity analysis was performed to validate the robustness of our results. The intercept of MR-Egger methods was used to test the horizontal pleiotropy and MR pleiotropy residual sum and outlier method (MR-PRESSO) was used to detect potential outliers[26]. In order to correct for multiple comparisons in multiple hypothesis, Benjamin-Hochberg procedure (FDR) was performed to correct for the number of exposures tested at each stage of microbiota and the significance of causal feature was set to $P_{FDR} < 0.10$. Microbial taxa were considered as potentially causal features if they reach the nominal significance of 0.05 with three main MR analysis but are not significant after FDR adjustment. Notably, three kinds of gut microbial taxa (*Methanobacteria* class, *Methanobacteriales* order and *Methanobacteriaceae* family) are exactly same, and therefore we only remained the result of *Methanobacteria* family. Furthermore, considering the critical impact of BMI on the progress from gut microbiota to the OA onset, we used a multivariable MR analysis to rule out the potential pleiotropy. Notably, SNPs related to both causal gut microbiota and BMI were utilized as IVs for multivariable MR analysis[27]. Then the multivariable MR analysis was performed to estimate the causal effect of gut microbes on OA after adjusting for BMI:

$$\widehat{\beta}^{OA} = \widehat{\beta}^{Microbes} a + \widehat{\beta}^{BMI} b + e,\ e \sim N\left(0,\ \sigma^2\right) \quad (1)$$

where $\widehat{\beta}$ represents the marginal effects of selected SNPs, and e denotes the residual error with variance σ^2. Then a and b can be estimated with the weighted least squares method. Additionally, reverse-direction MR analysis was conducted to examine whether there existed reverse-direction causal association.

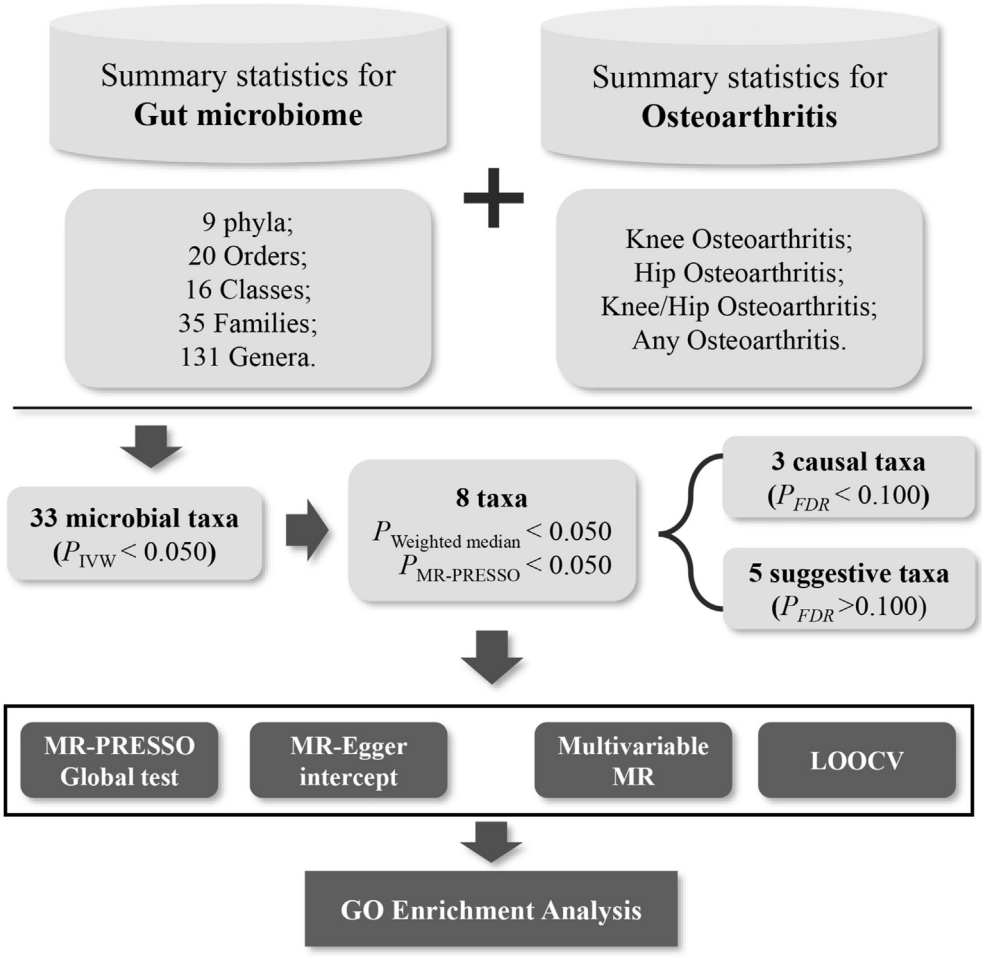

Fig. 1 Analysis Process of Our Research. MR, Mendelian Randomization; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; LOOCV, Leave-One-Out Cross-Validation; GO, Gene ontology.

GO enrichment analysis

To further explore the biological role of gut microbial taxa on the development of OA, we performed GO enrichment analysis based on lead SNPs for all identified gut microbial taxa. We mapped lead SNPs of causal microbial taxa identified in different OA phenotypes to the nearby genes.

All analysis were performed by using R software (version 3.5.3). 'MendelianRandomization' package (version 0.4.3) was used to perform MR analysis and multivariable MR analysis[28]. MR-PRESSO

Phenotypes		Sample size (case)	SNP	Reference
OA	Knee	455,221 (24,955)	35,874,742	PMID: pmid:30664745
	Hip	455,221 (15,704)	35,626,217	PMID: pmid:30664745
	Hip/Knee	455,221 (39,427)	36,164,724	PMID: pmid:30664745
	Any	455,221 (77,052)	37,106,466	PMID: pmid:30664745
Gut microbiota		18,340	5,717,754	PMID: pmid:33462485

Note: OA, osteoarthritis; GWAS, genome-wide association study; PMID, PubMed unique identifier.

Table I Detailed information of GWAS summary statistics used in our analysis

was conducted with 'MR-PRESSO' package[26]. GO enrichment analysis was performed by website tool "FUMA"[29].

Result

Overview

Based on IVW methods, we observed some significant evidence for the causal association between gut microbiota and OA risk. Finally, 31 microbial taxa were identified at significance of 0.05, including 1 class, 6 families, 21 genera, and 3 orders. Fig. 2 showed a panorama of potentially significant causal features and a total of 31 microbial taxa were significant for four different phenotypes of OA (Table II). However, after FDR correction ($P_{FDR} < 0.100$), 4 microbial taxa were remained for OA (3 of them for knee OA and 1 for any OA).

Causal effect of gut microbiota on knee OA

For knee OA, a total of 11 causal microbial taxa were identified, where 3 belongs to classes, 2 belongs to orders,3 belongs to families, and 5 belongs to genera. After FDR correction, five significant taxa were identified (i.e., *Methanobacteriaceae* family, *Desulfovibrionales* order and *Ruminiclostridium5* genus) (Fig. 3). Specifically, *Methanobacteriaceae* family is negatively associated with knee OA risk ($P_{FDR} = 0.043$). Based on three different methods, we observed consistent results ($P_{IVW} = 0.003$, $P_{Weighted\text{-}Median} = 0.013$, $P_{Mr\text{-}Presso} = 0.017$). The increase of *Desulfovibrionales* order could reduce the risk of knee OA ($P_{IVW} = 0.004$, $P_{FDR} = 0.045$), which is consistent to weighted-median ($P_{Weighted\text{-}Median} = 0.138$) and MR-PRESSO ($P_{Mr\text{-}Presso} = 0.042$). The causal effect of *Ruminiclostridium5* genus on knee OA is also significant with three different methods ($P_{IVW} = 4.82E\text{-}04$, $P_{FDR} = 0.063$, $P_{Weighted\text{-}Median} = 0.025$, $P_{Mr\text{-}Presso} = 7.66E\text{-}04$). Besides, one *unknown* genus coded as *ID 2071* reaches the nominal significance of 0.05 with three different methods ($P_{IVW} = 0.011$, $P_{Weighted\text{-}Median} = 0.012$, $P_{Mr\text{-}Presso} = 0.016$) and could reduce the OA risk (Fig. 3). Sensitive results proved the robustness of the causal associations between gut microbiota for OA (Table S2). However, intercept of MR-Egger showed potential horizontal pleiotropy for the causal effect of *Methanobacteriaceae* family on OA. By carefully searching for GWAS catalog, one potentially pleiotropic SNP (i.e., rs11018665 located in NOX4) was observed which participated in several complex diseases. After removing it, the result of causal association is still stable

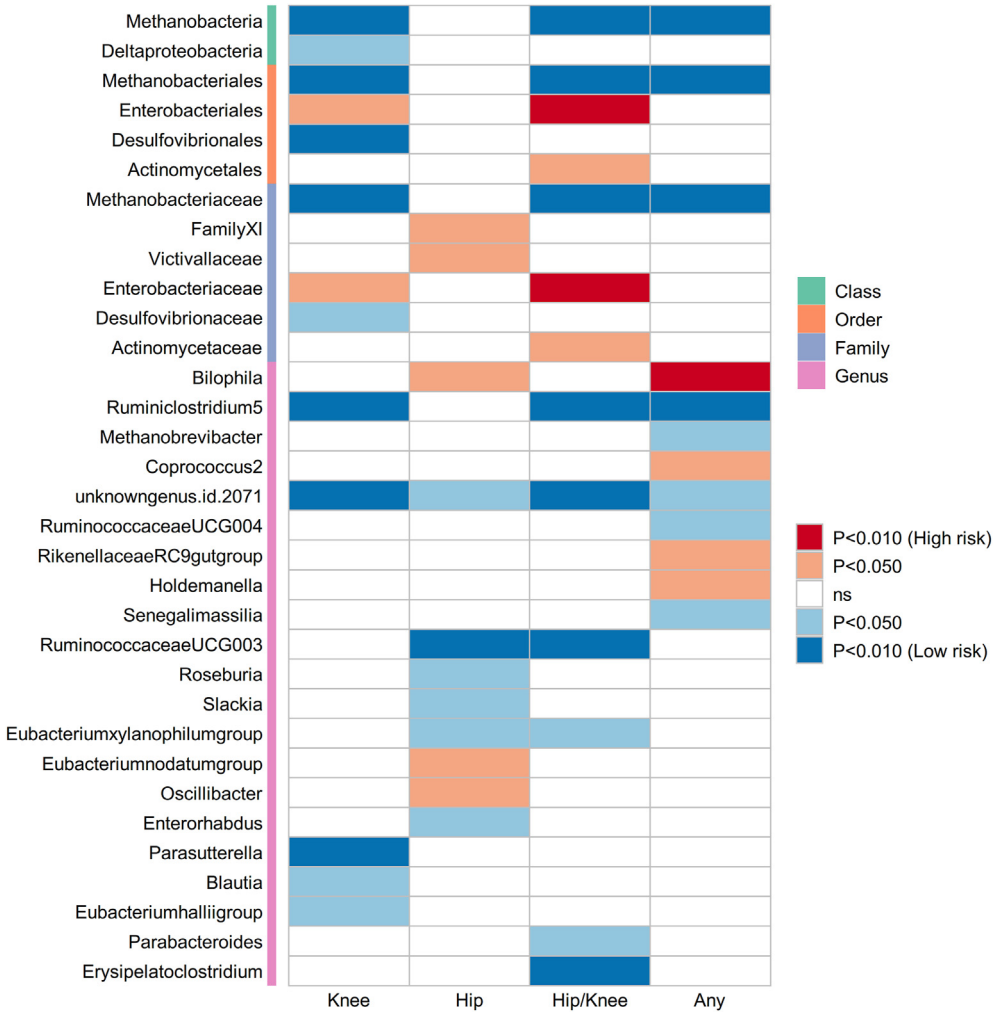

Fig. 2 Causal effect of gut microbial taxa on OA identified at the nominal significance (***P*** < 0.05/0.01). Note: Knee represents OA (knee), Hip represents OA (hip), Hip/Knee represents OA (hip/knee), Any represents OA (Any); OA, osteoarthritis.

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(P_{IVW} = 0.018, $P_{Weighted\text{-}Median}$ = 0.019, $P_{Mr\text{-}Presso}$ = 0.017), but the horizontal pleiotropy disappeared ($P_{Egger\text{-}intercept}$ = 0.192). Besides, global test of MR-PRESSO were performed to rule out the possibility of horizontal pleiotropy ($P_{Global\text{-}test}$ = 0.413 for *Methanobacteriaceae* family, $P_{Global\text{-}test}$ = 0.134 for *Desulfovibrionales* order, $P_{Global\text{-}test}$ = 0.844 for *Ruminiclostridium5* genus and $P_{Global\text{-}test}$ = 0.844 for *Ruminiclostridium5* genus). We also drew scatter plots and funnel plots for causal and suggestive microbial taxa, which showed that no potential outlier could affect our estimation substantially (Fig. 4 and Figs. S1 and S2). LOOCV results showed that no SNP with large effect size could bias our estimation (Fig. S3).

Causal effect of gut microbial taxa on hip OA

For Hip represents OA (hip) OA, a total of 11 causal microbial taxa were identified, where 2 belongs to families, 9 belongs to genera. After FDR adjustment, no microbial taxa were significant. However, two microbial taxa that passed three different MR methods could be viewed as suggestively causal features (Fig. 3 and Figs. S1 and S2). The decrease of hip OA risk could attribute to the increase of *RuminococcaceaeUCG003* genus (P_{IVW} = 0.008, $P_{Weighted\text{-}Median}$ = 0.004, $P_{Mr\text{-}Presso}$ = 0.002) and *Enterorhabdus* genus (P_{IVW} = 0.039, $P_{Weighted\text{-}Median}$ = 0.037, $P_{Mr\text{-}Presso}$ = 0.019). Sensitive results ruled out the potential heterogeneity and horizontal pleiotropy in causal associations by using global test of MR-PRESSO and intercept of MR-Egger (Table S2). LOOCV results showed that no SNP with large effect size could bias the estimation of *RuminococcaceaeUCG003* genus but could interfere *Enterorhabdus* genus (Fig. S3). Global test of MR-PRESSO ruled out the possibility of horizontal pleiotropy ($P_{Global\text{-}test}$ = 0.926 for *RuminococcaceaeUCG003* genus and $P_{Global\text{-}test}$ = 0.865 for *Enterorhabdus* genus). Scatter plots and funnel plots observed that no potential outlier could affect the causal association (Figs. S1 and S2).

Causal effect of gut microbial taxa on hip/knee and any OA

Furthermore, we identified a total of 10 microbial taxa for any OA and 11 for hip/knee OA at the nominal significance ($P < 0.05$). Only *Methanobacteriaceae* family (P_{FDR} = 0.013) was significant after FDR adjustment and consistent results were observed for three MR methods (P_{IVW} = 8.14E-04, $P_{Weighted\text{-}Median}$ = 0.025, $P_{Mr\text{-}Presso}$ = 0.004) (Figs. 3 and 4). Global test of MR-PRESSO were performed to rule out the possibility of horizontal pleiotropy ($P_{Global\text{-}test}$ = 0.655). Four microbial taxa were identified to be suggestively associated with any and hip/knee OA (i.e., *Ruminiclostridium5* genus, *RuminococcaceaeUCG003* genus, *RuminococcaceaeUCG004* genus and *unknown* genus *id:2071*) (Fig. 3 and Figs. S1 and S2). Notably, consistent with knee OA, the increase in abundance of *Ruminiclostridium5* genus could contribute to the decrease of any OA risk (P_{IVW} = 0.008, $P_{Weighted\text{-}Median}$ = 0.044, $P_{Mr\text{-}Presso}$ = 0.012) and hip/knee OA risk (P_{IVW} = 0.002, $P_{Weighted\text{-}Median}$ = 0.026, $P_{Mr\text{-}Presso}$ = 0.003). Additionally, suggestively causal effects of two

Group	Gut microbiota	Traits 1	Traits 2	N	OR (95%CI)	*p*
Class	Deltaproteobacteria	Knee		13	0.895 (0.819, 0.978)	0.014
Order	Actinomycetales	Hip/Knee		5	1.087 (1.001, 1.180)	0.048
Order	Desulfovibrionales	Knee		12	0.876 (0.800, 0.960)	0.004
Order	Enterobacteriales	Hip/Knee	Knee	11	1.113 (1.026, 1.207)	0.010
Family	Actinomycetaceae	Hip/Knee		5	1.086 (1.001, 1.179)	0.048
Family	Desulfovibrionaceae	Knee		10	0.907 (0.823, 1.000)	0.050
Family	Enterobacteriaceae	Hip/Knee	Knee	11	1.113 (1.026, 1.207)	0.010
Family	FamilyXI	Hip		10	1.065 (1.002, 1.133)	0.043
Family	Methanobacteriaceae	Any	Knee, Hip/Knee	11	0.951 (0.923, 0.979)	0.001
Family	Victivallaceae	Hip		14	1.064 (1.007, 1.124)	0.026
Genus	Bilophila	Any, Hip		16	1.069 (1.020, 1.121)	0.005
Genus	Blautia	Knee		13	0.910 (0.832, 0.995)	0.039
Genus	Coprococcus2	Any		11	1.063 (1.008, 1.122)	0.025
Genus	Enterorhabdus	Hip		7	0.899 (0.813, 0.995)	0.039
Genus	Erysipelatoclostridium	Hip/Knee		15	0.924 (0.874, 0.978)	0.006
Genus	Eubacteriumhalliigroup	Knee		16	0.913 (0.843, 0.988)	0.025
Genus	Eubacteriumnodatumgroup	Hip		11	1.064 (1.002, 1.130)	0.042
Genus	Eubacteriumxylanophilumgroup	Hip/Knee	Hip	11	0.908 (0.842, 0.981)	0.014
Genus	Unknowngenus id:2071	Hip/Knee	Knee, Any, Hip	19	0.915 (0.862, 0.970)	0.003
Genus	Holdemanella	Any		12	1.040 (1.001, 1.081)	0.042
Genus	Methanobrevibacter	Any		7	0.958 (0.922, 0.994)	0.023
Genus	Oscillibacter	Hip		15	1.100 (1.014, 1.193)	0.022
Genus	Parabacteroides	Hip/Knee		10	0.909 (0.833, 0.991)	0.031
Genus	Parasutterella	Knee		15	0.911 (0.849, 0.978)	0.010
Genus	RikenellaceaeRC9gutgroup	Any		13	1.029 (1.002, 1.057)	0.038
Genus	Roseburia	Hip		14	0.883 (0.788, 0.989)	0.032
Genus	Ruminiclostridium5	Knee	Any, Hip/Knee	15	0.847 (0.771, 0.93)	0.000
Genus	RuminococcaceaeUCG003	Hip/Knee	Hip	13	0.895 (0.829, 0.966)	0.004
Genus	RuminococcaceaeUCG004	Any		11	0.947 (0.900, 0.997)	0.036
Genus	Senegalimassilia	Any		8	0.953 (0.908, 1.000)	0.048
Genus	Slackia	Hip		8	0.899 (0.816, 0.989)	0.029

Note: If microbiota is significant across different phenotypes, we only present the association with smallest *P* values. OR, odds ratio; CI, confidence interval; OA, osteoarthritis; IVW, inverse-variance weighted.

Table II Summary of causal association between lead gut microbiota and OA risk by using IVW method

Ruminococcaceae genus were determined: i.e., *Ruminococcaceae-UCG003* genus for hip/knee OA (P_{IVW} = 0.004, $P_{Weighted\text{-}Median}$ = 0.023, $P_{Mr\text{-}Presso}$ = 0.015) and *Ruminococcaceae-UCG004* genus for any OA (P_{IVW} = 7.94E-04, $P_{Weighted\text{-}Median}$ = 0.007, $P_{Mr\text{-}Presso}$ = 0.015) (Fig. 3). Besides, *unknown* genus *id:2071* was also potentially significant for hip/knee OA (P_{IVW} = 0.003, $P_{Weighted\text{-}Median}$ = 0.004, $P_{Mr\text{-}Presso}$ = 0.007), which is consistent with knee OA. The causal effect of lead gut microbial taxa on OA risk was summarized in Table II. Sensitive results ruled out the potential heterogeneity and horizontal pleiotropy in causal associations by using global test of MR-PRESSO and intercept of MR-Egger. Scatter plots and funnel plots excluded the potential effect of outlier (Fig. 4 and Figs. S1 and S2). LOOCV results gave the evidence that our estimation could not be interfered by SNP with large effect size (Fig. S3).

Multivariable MR analysis

To further examine whether horizonal pleiotropy could essentially change our results, we performed multivariable MR analysis to adjust for BMI. Results ruled out the possibility of potentially pleiotropic effect after adjusting for BMI (Fig. 3). For knee OA, the independently causal effect was estimated to be 0.916 (95%CI: 0.850–0.987, P_{mvmr} = 0.021) for *Methanobacteriaceae* family on knee OA, where similar results were observed for *Desulfovibrionales* order on knee OA (OR = 0.854, 95%CI: 0.734–0.994, P_{mvmr} = 0.042) and *Ruminiclostridium5* genus (OR = 0.846, 95%CI: 0.739–0.969, P_{mvmr} = 0.016). Besides, the causal effect was estimated to be 0.951 (95%CI: 0.908–0.997, P_{mvmr} = 0.039) for *Methanobacteriaceae* family on any OA. However, the multivariable results for suggestive microbial taxa turned to be insignificant after adjustment, indicating a potential pleiotropic effect of BMI on gut microbial taxa and development of OA (Fig. 3).

Reverse-direction MR analyses

Finally, reverse MR analysis gave the evidence that there is not causal effect of OA on 8 gut microbial taxa (Table S3). However, only for the association between any OA with *Ruminiclostridium5* genus, a potential significant causal effect was observed (P_{IVW} = 0.015, $P_{Weighted\text{-}Median}$ = 0.110, $P_{Mr\text{-}Presso}$ = 0.045).

GO enrichment analysis

GO terms enrichment analysis of microbial taxa on different OA found significant enrichment of several crucial regulation pathways. For knee OA, 49 GO biological processes (e.g., the regulations of melanocyte, pigment cell, and multicellular organism process) were observed to be involved in OA (Fig. S4). For hip OA, 3 GO biological processes were observed to be involved in OA (Fig. S5), i.e., regulation of membrane potential, response to ammonium ion, and positive regulation of voltage-gated potassium channel activity. A total of 137 GO biological processes were found to participate in hip/knee OA (e.g., positive regulation of multicellular organic processes and nervous system development regulation) and we only presented the top 40 terms in Fig. S6. Finally, for any OA, only 5 GO terms were determined (e.g., nervous system development regulation and positive regulation of multicellular organic processes) (Fig. S7).

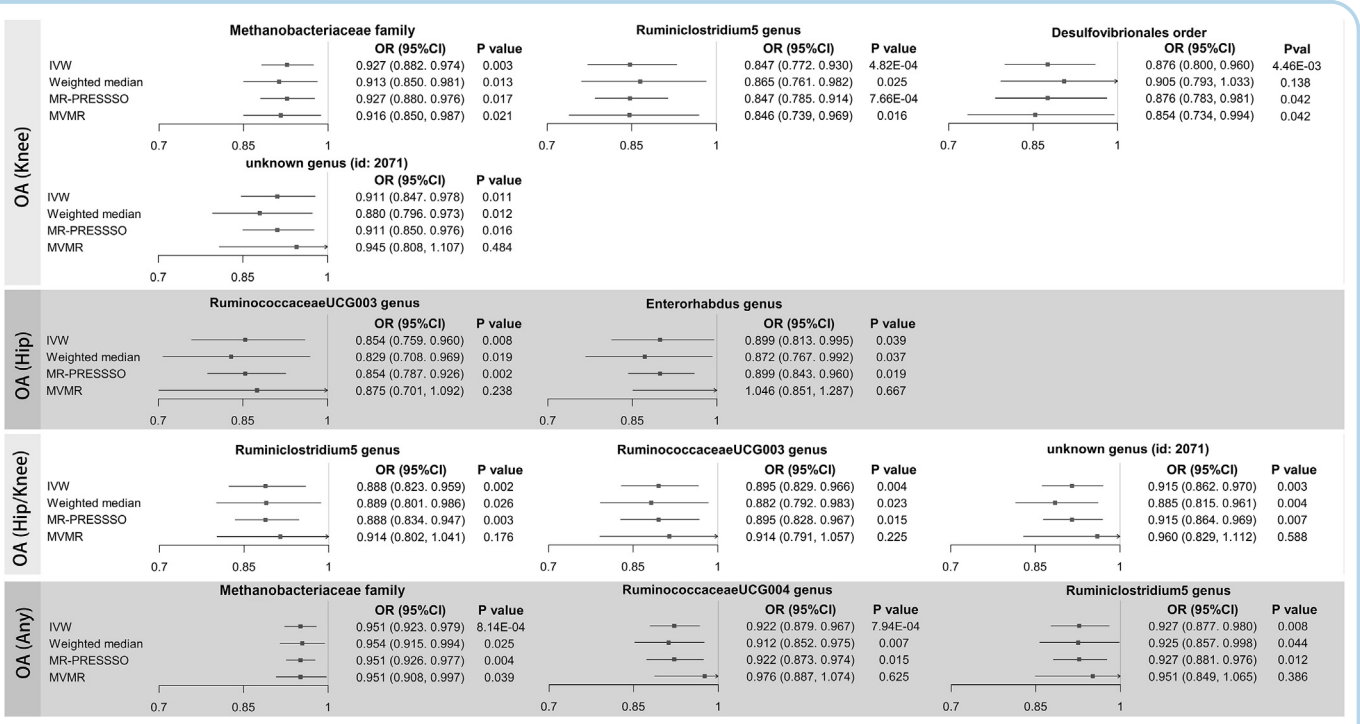

Fig. 3

Forest plot for causal association between 8 unique causal/suggestive microbial taxa with OA. OR, odds ratio; CI, confidence interval; OA, osteoarthritis; IVW, inverse-variance weighted, MVMR, multivariable variable Mendelian randomization. For OA (Knee), three taxa are causal (i.e., Methanobacteriaceae family, Ruminiclostridium5 genus and Desulfovibrionales order); For OA (Any), 1 taxon is causal (Methanobacteriaceae family).

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Discussion

To our knowledge, this is the first time to investigate the causal associations comprehensively and deeply between gut microbiota and OA based on publicly available genetic databases. This study conducted comprehensive MR analyses for 211 taxa based on 18,340 individuals (24 cohorts) to reveal the potential role of gut microbiota in the development of OA. Findings of our research suggested that multiple gut microbial taxa play crucial roles in the development of OA, including 3 causal microbial taxa (i.e., *Methanobacteriaceae* family, *Desulfovibrionales* order, *Ruminiclostridium5* genus) and 5 suggestive microbial taxa.

An experimental study observed a decrease in *Ruminiclostridium* 5 genus in rats with collagen-induced arthritis (CIA), suggesting a potential link between *Ruminiclostridium* 5 and the development of OA[30].Other studies have shown a positive correlation between *Ruminiclostridium* and vitamin D3 abundance, and furthermore, vitamin D3 deficiency may be a risk factor for OA in clinical practice[31,32].Based on our results, *Ruminiclostridium* has a protective effect against OA, suggesting possible mechanisms by which *Ruminiclostridium* is involved in regulating the progression of OA by affecting vitamin D3 metabolism. Our comprehensive MR analyses showed protective effect of *Methanobacteriaceae* family on OA risk, whereas the impact of methanogenic bacteria in gut microbiota on OA development is unknown until now. Different from our results, a rat experiment found the presence of *Methanobacteriaceae* family in DIO rats was positively correlated with pro-inflammatory factors in Mankin Scores, serum and synovial fluid[33]. Furthermore, we found that *Desulfovibrionales* order has a positive effect on OA of the knee. A 16S rRNA sequencing on mice observed a less abundance of *Desulfovibrionales* order in susceptible to CIA mice, compared to CIA-resistant mice before onset of OA[34]. Additionally, *Desulfovibrionales* has been proved to be involved in immune activity. Experiment observed that the abundance of family *Desulfovibrionaceae* (belong to *Desulfovibrionales* order) in Interleukin-1α (IL-1α) knockout mice is considerably higher than control group, implying relationship between IL-1 and *Desulfovibrionales* order[35]. IL-1 has been proved to play a key role in the development of OA due to that it can independently induce inflammatory responses and catabolic effects and binds to articular cartilage and other mediators[36,37].

Several suggestive gut microbial taxa were also detected in the MR analysis, some of which have been proved in previous observational studies. As anaerobic bacteria, *Ruminococcaceae* has been proved to be negatively correlated with the OARSI OA cartilage histopathology assessment system scores[38]. *Ruminococcaceae* showed a significantly negative correlation with pro-inflammatory factors (e.g., IL-1β, IL-6 and MIP-1α). Increased levels of pro-inflammatory factors induce the activation of signaling pathways, which in turn produces more inflammatory molecules and eventually lead to the degradation of the extracellular matrix and change the anatomical and physiological functions of the joints[39,40]. CIA-susceptible mice had less abundance of *Enterobacter* genus before arthritis onset than mice resistant to CIA. But after the onset of arthritis, the number of *Enterobacter* genus in CIA-susceptible mice increased sharply as the arthritis progressed[34]. Additionally, studies have shown that the abundance of *Enterorhabdus* genus is negatively correlated with adiponectin and that adiponectin is

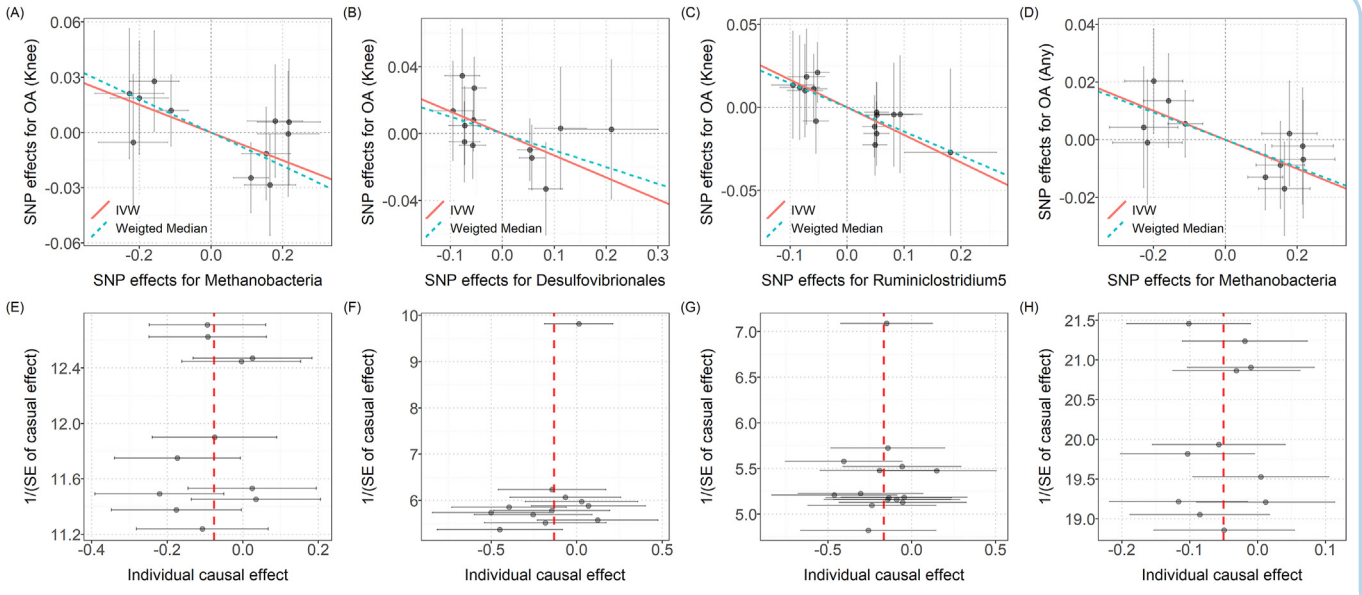

Fig. 4

Causal association between causal microbial taxa with OA. In A-D, red lines represent estimations with IVW method and green dotted lines represent estimations with weighted median method. In E-F, red dotted lines represent the estimations of all SNPs. (A) Scatter plot for causal effect of *Methanobacteria* family on OA (knee); (B) Scatter plot for causal effect of *Desulfovibrionales* order on OA (knee); (C) Scatter plot for causal effect of *Ruminiclostridium5* genus on OA (knee); (D) Scatter plot for causal effect of *Methanobacteria* family on OA (Any); (E) Funnel plot for causal effect of *Methanobacteria* family on OA (knee); (F) Funnel plot for causal effect of *Desulfovibrionales* genus on OA (knee); (G) Funnel plot for causal effect of *Ruminiclostridium5* genus on OA (knee); (H) Funnel plot for causal effect of *Methanobacteria* family on OA (Any). OA, osteoarthritis; IVW, inverse-variance weighted.

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associated with cartilage destruction in osteoarthritis and may mediate the destruction of cartilage[41], which supports the validity of our results[42]. Interestingly, an *unknown* genus which is coded as 2071 is significance in four OA phenotypes at a nominal significance level. However, its specific information and biological function is still unknown, and further research and discoveries are needed. Additionally, a large population-based cohort study observed that numerous *Streptococcus* species were associated with increased knee pain, but our MR results did not support this association[43]. We believe that this inconsistency is due to the unavoidable confounding factors in population studies, which are very common in microbiome studies.

Furthermore, GO enrichment analysis found that larger number of GO biologic processes play key role in relationship between gut microbiota and OA, which have been supported by previous studies. For example, regulation of melanocytes was observed to be involved in the effects of intestinal microbes on knee, hip and hip/knee OA, which has been proved that the can secrete pro-inflammatory cytokines, participate widely in anti-inflammatory[44]. Neuronal damage can be considered as another risk factor for the development and onset of OA in addition to the common risk factors[45], where nervous system development was also observed to be enriched in our analysis. Several evidence have suggested that cell death is a central feature of cartilage degeneration in osteoarthritis, and our enrichment analysis shows that intestinal flora is negative for cell death[46–48]. A mouse experiment showed that multiple injections of transforming growth factor beta into the knee joint induced changes in articular cartilage and surrounding tissues that were very similar to those characteristic of spontaneous arthritis, suggesting the key role of TGF-β in the development of OA[49]. Elevated levels of TGF-β are the primary cause of OA synovial fibrosis[50]. Recent studies have shown that inhibition of TGF-β activity in subchondral bone results in reduced degeneration of articular cartilage, but inhibition of TGF-β activity has a detrimental effect on cartilage homeostasis, suggesting that the effect of TGF-β on OA is multifaceted[51]. We speculate that intestinal flora may affect OA by influencing TGF-β.

It is worth noting that obesity-mediated changes in the gastrointestinal microbiome can cause systemic and local low-grade inflammation, finally leading to OA onset[15,52]. Our multivariable MR analysis ruled out the pleiotropic effects of BMI exist between causal gut microbiota and development of OA. The statistically insignificant results for suggestive gut microbiota could attribute to limited statistical power or potential pleiotropic effects of BMI. Interestingly, our results also found that influence of gut microbiota on knee and hip OA are exactly different, suggesting the specificity of OA onset among joint sites. Only few studies focused on the relationship between gut microbes and the risk of OA in different joint sites. In line with our results, Dunn *et al.* gave the evidence that gut microbes were distinct in knee and hip cartilage, both with and without OA[53].

In conclusion, our study reported an unprecedented comprehensive screening of gut microbes associated with OA, and our results also found a protective role of multiple gut microbiota in the development of OA, providing guidance for the prevention and treatment of OA in clinical practice. However, there are also a few limitations to our study: First, although we have identified these meaningful gut microbiota, further studies are needed to reveal their role in the pathogenesis of OA. Second, limited sample size for gut microbiota could bias our estimation to some extent and further expansion of the sample size may give us a more accurate estimate of the relationship between gut microbiota and OA. Third, although the majority of the data used in our study were European, a small number of the microbiological data were of other races, which may confound our estimates to some extent. Finally, due to the lack of individual data, we were unable to conduct further population stratification studies (e.g., gender) and explore possible differences in different populations.

Data sharing statement

Data are available in a public, open access repository. Data URLs: https://molgenis135.gcc.rug.nl/; https://www.ebi.ac.uk/gwas/down-loads/summary-statistics.

Author contributions

SL and XY conceived the design of the study; XY and YY obtained the data; YY and XY cleared up the datasets; YY and XY mainly performed the data analyses; SL, LB, YY, XY and RC drafted and revised the manuscript, and all authors approved the manuscript and provided relevant suggestions.

Conflict of interest

None declared.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2021.08.003.

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