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Causal relationships between gut microbiota, plasma metabolites, and HIV infection: insights from Mendelian randomization and mediation analysis

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Abstract

Objective Gut dysbiosis and metabolic abnormalities have been implicated in HIV infection. However, the exact causal relationships among the gut microbiota, metabolites, and HIV infection remain poorly understood. Our study involving Mendelian randomization (MR) and mediation analysis aims to unveil these causalities.

Methods Genetic instrumental variables for the gut microbiota were retrieved from MiBioGen consortium (n = 18,340). Metabolism-related genetic variants were sourced from the CLSA cohort (n = 8299). GWAS summary statistics for symptomatic HIV infection were derived from the FinnGen study (n = 309,154), and the UK Biobank (n = 208,808). We performed the bidirectional two-sample MR to assess causalities with the inverse-variance weighted (IVW) method as the primary analysis. Moreover, we executed a mediation analysis using two-step MR methods.

Results Compared to the causal effects of HIV infection on gut microbiota (or metabolites), those of gut microbiota (or plasma metabolites) on the risk of HIV infection were more substantial. Phylum *Proteobacteria* (OR: 2.114, 95% CI 1.042–4.288, $P = 0.038$), and genus *Ruminococcaceae UCG013* (OR: 2.127, 95% CI 1.080–4.191, $P = 0.029$) exhibited an adverse causal effect on HIV infection, whereas genus *Clostridium sensu stricto 1*(OR: 0.491, 95% CI 0.252–0.956, $P = 0.036$) and family *Erysipelotrichaceae* (OR: 0.399, 95% CI 0.193–0.827, $P = 0.013$) acted as significant protective factors for HIV. The salicyluric glucuronide level (OR = 2.233, 95% CI 1.120–4.453, $P = 0.023$) exhibited a considerably adverse causal effect on HIV infection. Conversely, the salicylate-to-citrate ratio (OR: 0.417, 95% CI 0.253–0.688, $P = 0.001$) was identified as a protective factor for HIV. We identified only one bidirectional causality between 1-palmitoyl-GPI and HIV infection. Mechanistically, genus *Haemophilus* mediated the causal effects of three phospholipids on HIV infection risk: 1-palmitoyl-GPI (mediation proportion = 33.7%, $P = 0.018$), 1-palmitoyl-2-arachidonoyl-GPI (mediation proportion = 18.3%, $P = 0.019$), and 1-linoleoyl-2-linolenoyl-GPC (mediation proportion = 20.3%, $P = 0.0216$). Additionally, 5-Dodecenoylcarnitine (C12:1) mediated the causal effect of genus *Sellimonas* on the risk of HIV infection (mediation proportion = 13.7%, $P = 0.0348$).

Conclusion Our study revealed that gut microbiota and metabolites causally influence HIV infection risk more substantially than the reverse. We identified the bidirectional causality between 1-palmitoyl-GPI (16:0) and HIV infection,

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and elucidated four mediation relationships. These findings provide genetic insights into prediction, prevention, and personalized medicine of HIV infection.

Keywords HIV, AIDS, Mendelian randomization, Plasma metabolites, Gut microbiota, Meta-analysis

Introduction

Since the first cases of AIDS were reported more than 40 years ago, approximately 84 million people worldwide have been infected with HIV-1, 40 million of whom have died from AIDS-related illnesses [1]. The hallmark of HIV-1 infection is the depletion of CD4+ T cells, rendering the affected individuals susceptible to opportunistic infections and cancers [1]. HIV-1 infection activates both the mucosal and systemic immune systems, which, in turn, cause alterations and translocations of the gut microbiome [2–4]. Bacterial 16S ribosomal RNA gene sequencing of gut microbiota from individuals with HIV-1 infection revealed higher levels of *Proteobacteria* [2, 3] and lower levels of *Firmicutes* [2]. Additionally, the structure of the *Bacteroidetes* bacterial community was significantly altered [2, 4]. The intestinal dysbiosis observed in HIV-1 infection is associated with elevated levels of interleukin-1β, interferon-γ, tumor necrosis factor-α, sCD14 [3], and the activation ofT cells and myeloid dendritic cells [2]. Although there is some consistency in research findings as previously mentioned, conclusions about changes in gut microbiota abundance vary to some extent across studies. For instance, the abundance of *Barnesiella* was found to be elevated in HIV patients in the study by Dinh et al. [3], while it was found to be decreased in the study by Dillon et al. [2]. Notably, various studies on HIV infection have shown that gut microbiota is influenced by confounding factors such as age, race, diet, medications, and individual behaviors [5], resulting in considerable variations in research results.

The interplay between gut dysbiosis and metabolic abnormalities has been implicated in various pathological states. Gut microbiota-derived metabolites impact immune responses in infectious diseases [6], and participate in the development of diabetes mellitus [7] and metabolic syndrome [8]. Moreover, gut dysbiosis facilitates the development of some cardiovascular diseases [9, 10] via various metabolic pathways. Research on the effect of HIV infection on gut microbiota and metabolites is underway. Imidazole-propionate, a gut microbiota-derived metabolite, has been reported to be positively associated with carotid artery atherosclerosis in women with HIV infection [11]. However, the bidirectional crosstalk between gut microbiota and metabolites in the context of HIV infection is not yet fully understood.

Mendelian randomization (MR) is an inference approach to assess causal relationships between exposures and disease outcomes by using naturally occurring genetic variations as instrumental variables (IVs) for exposure factors [12, 13]. Mendelian randomization analysis are based on three core hypotheses: relevance, independence, and exclusion restriction [14]. It is assumed that the IVs are strongly related to the exposure factors (relevance), but not with any confounders (independence), and that the IVs are not directly correlated with the outcome via any pathways other than the exposure of interest (exclusion restriction). As genetic variations (single-nucleotide polymorphisms [SNPs]) are randomly allocated independent of environmental factors, MR can eliminate the influence of confounding factors [12, 15, 16]. Also, MR prevents reverse causality, as genetic variations precede clinical outcomes and disease progression [12, 16]. Compared to randomized clinical trials (RCTs), Mendelian randomization studies avoid issues such as high costs, extensive follow-up time, limited sample sizes, reverse causality, confounding and ethical approval [17]. Thus, MR affords practicality that cannot be attained by clinical research studies, and offers robust evidence of causal effects.

Currently, MR studies on HIV/AIDS are still in the early stages. So far, only two MR studies on HIV infection are available. One of them elucidates the mediating role of DNA methylation in the causal association between cocaine use and HIV severity [18], while the other explores the effects of protein biomarkers on cardiovascular diseases in individuals with HIV [19]. To comprehensively explore the causalities among gut microbiota, plasma metabolites, and HIV infection, we performed a bidirectional two-sample MR using genome-wide association study (GWAS) data. Furthermore, we conducted mediation analyses to clarify the crosstalk between gut microbiota and metabolites in the development of HIV infection.

Methods

Study design

This study used bidirectional two-sample MR to assess the causal relationships among gut microbiota, metabolites, and HIV infection. Sensitivity analysis was used to examine the robustness and credibility of causal estimates. A combined analysis of MR results was conducted using meta-analysis. Furthermore, a mediation model

for metabolites (microbiota) to assess the causal effects of microbiota (metabolites) on HIV infection was developed. Figure 1 illustrates the overall design of this study.

Data resources

Human gut microbiota-related SNPs were retrieved from the international consortium initiative MiBioGen (https://mibiogen.gcc.rug.nl/menu/main/home), which curated and analyzed data on 16S rRNA microbiome and genotypes from 18,340 European-dominated participants. [20]. Metabolism-related genetic variants were obtained from the GWAS conducted on the Canadian Longitudinal Study on Aging (CLSA) cohort (https://www.ebi.ac.uk/gwas/publications/36635386), which included 1091 plasma metabolites and 309 metabolite ratios analyzed in 8299 participants of European ancestry [21]. The only two publicly available GWAS summary statistics for symptomatic HIV infection were extracted from the FinnGen consortium R7 release data [22] (https://r7.risteys.finngen.fi/phenocode/AB1_HIV) and the UK Biobank (UKB) data [23, 24] (https://www.ebi.ac.uk/gwas/studies/GCST90041717). The FinnGen study included 309,154 samples from the Finnish population. The dataset from the UKB consists of 208,808 samples of European ancestry. This study has been conducted using publicly available GWAS summary statistics. Ethical approval and participant consent were obtained in the original studies [20–24].

IV selection

To select strong IVs for gut microbiota and HIV, given that very few SNPs (for gut microbiota) or no SNP (for HIV) association reached the genome-wide significant threshold ($P<5\times10^{-8}$), a compromised significant level ($P<1\times10^{-5}$ for gut microbiota [12, 25], $P<5\times10^{-6}$ for HIV [18]) was used to extract IVs. European 1000 Genome Project was used to determine the linkage disequilibrium (LD) between all SNPs (clumping window size = 10,000 kb, $r^2<0.001$) [12, 26, 27]. SNPs with minor allele frequency (MAF) ≤ 0.01 were excluded [12]. Harmonizing processes were conducted to exclude SNPs for being palindromic with intermediate allele frequencies. Approximated F-statistics were applied to assess the instrument strength and eliminate the bias originating from weak IVs using the following formula [12]:

$$F = R^2 \times (n-\kappa-1)/\kappa \times (1-R^2)$$

R^2 represents the exposure variance interpreted by the selected SNPs, n represents the sample size, and k represents the number of IVs. SNPs with F-statistics of > 10 were selected [27]. The Steiger filtering analysis was utilized to infer the direction of causality [28]. Prior to conducting the MR analysis, SNPs with a stronger association with the outcome than with the exposure were filtered out using the Steiger test [28].

To select strong IVs for plasma metabolites, we initially chose SNPs that strongly and independently (clumping window size = 10,000 kb, $r^2<0.001$; F-statistics > 10; MAF > 0.01) predicted metabolites at genome-wide significance ($P<5\times10^{-8}$). Out of the 1400 metabolites in the CLSA study, only 425 metabolites had strong IVs. As the majority of the metabolites had no or limited (< 3) SNPs at $P<5\times10^{-8}$, a less stringent significance threshold ($P<5\times10^{-6}$) was employed for selecting IVs [29, 30]. SNPs significantly associated with potential confounders were examined and excluded using the PhenoScanner GWAS database [31].

Bidirectional two-sample MR analysis

The pairwise causal effects among gut microbiota, metabolites, and HIV infection were assessed using inverse variance weighting (IVW) as the primary MR analysis method. The other four robust methods (MR-Egger, weighted median, simple mode, and weighted mode) were used for complementary analyses. The MR analyses were carried out by using the "TwoSampleMR" (version 0.5.6) package [32] in R software (version 4.3.1). Statistical significance for the causal effect was considered at $P<0.05$.

Sensitivity analysis

To detect the degree of heterogeneity, Cochrane's Q test was employed [30, 33]. $P>0.05$ indicates the absence of significant heterogeneity among the estimates from each SNP. MR-PRESSO and MR-Egger regression were employed to examine the possible horizontal pleiotropy effect [30, 33] ($P<0.05$ was judged significant). We further removed the MR results with pleiotropic effects and retained the remaining results for meta-analysis. We performed sensitivity analyses using the R package "MR-PRESSO" (version 1.0) [34] in R software (version 4.3.1).

Combined analysis

We conducted a meta-analysis to combine the MR results deriving from two HIV-related GWAS databases (FinnGen and the UKB). The selection of the meta-analysis model depends on the presence or absence of heterogeneity. In the absence of heterogeneity ($P>0.05$ for the Q test and $I^2\leq50\%$), the meta-analysis is conducted using a fixed effect model. Conversely, a random effect model is selected for the meta-analysis when the Q-value is significant ($P<0.05$) or $I^2>50\%$, indicating the presence of heterogeneity across studies [35]. We performed the meta-analysis using the R package "meta" (version 6.5-0) [36]. $P<0.05$ was considered statistically significant.

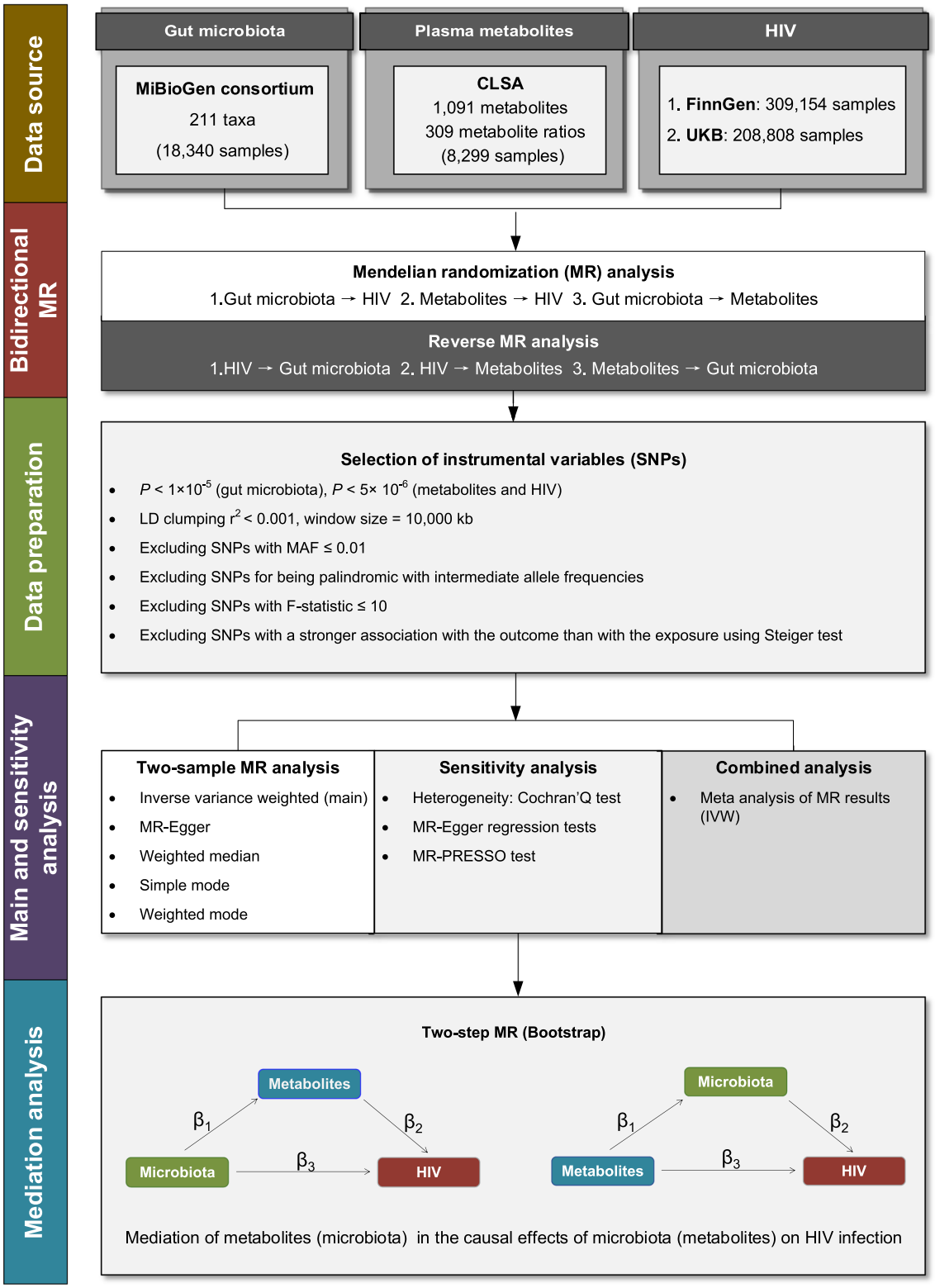

Fig. 1 Study design. CLSA, the Canadian Longitudinal Study on Aging Cohort; HIV, human immunodeficiency virus; LD, linkage disequilibrium; MAF, minor allele frequency; MR, Mendelian randomization; SNPs, single-nucleotide polymorphisms; UKB, the UK Biobank

Mediation analysis

We conducted mediation analysis using two-step MR to estimate the indirect effect of metabolites (microbiota) as mediators in the causal effects of microbiota (metabolites) on HIV infection. Two-step MR involves the computation of two MR estimates: (1) the causal effect of the exposure on the mediator (β_1), and (2) the causal effect of the mediator on the outcome (β_2) [13]. The indirect effect of the mediator(s) can then be calculated by the product of these two estimates ($\beta_1 \times \beta_2$) [13]. Here, β_3 represents the total effect of exposure on the outcome based on the two-sample MR analysis. The mediation proportion was calculated by dividing the indirect effect by the total effect ($\frac{\beta 1 \times \beta 2}{\beta 3}$) [13, 37]. The Bootstrap method was used to estimate the significance of the product of coefficients [38]. $P < 0.05$ was considered statistically significant.

Results

Causality between HIV infection and gut microbiota (or plasma metabolites)

Bidirectional MR results on HIV infection and gut microbiota (or plasma metabolites) are reported in Figs. 2 and 3, Supplementary Figures S1–S6, and Supplementary Tables S1–S12. Cochran's Q statistics revealed no significant heterogeneity across single instrument effects within each database (Supplementary Figures S1, S2, S4 and S6). After removing MR results with pleiotropic effects ($P < 0.05$ for MR-PRESSO or MR-Egger), we conducted meta-analyses to integrate the results of bidirectional MR analyses based on two HIV-related GWAS summary statistics from the FinnGen consortium and UKB datasets, as shown in Figs. 2 and 3, Supplementary Figures S3 and S5, and Supplementary Tables S3, S6, S9, and S12.

Causal effects of HIV on gut microbiota

The meta-analysis of the primary MR analysis (IVW) yielded evidence supporting the causal effects of HIV infection on nine bacterial taxa (Fig. 2, Supplementary Figure S1, and Supplementary Tables S1–S3). HIV infection could increase the abundance of three phylum *Bacteroidetes* bacterial taxa, including: phylum *Bacteroidetes* (β: 0.017, 95% confidence interval [CI] 0.004–0.030, $P = 0.01$), class *Bacteroidia* (β: 0.016, 95% CI 0.003–0.029, $P = 0.014$), order *Bacteroidales* (β: 0.016, 95% CI 0.003–0.029, $P = 0.014$), Additionally, an increase was observed in one *Proteobacteria* phylum bacterial taxon, genus *Sutterella* (β: 0.026, 95% CI 0.009–0.043, $P = 0.003$). Conversely, HIV infection could decrease the population of five phylum *Firmicutes* bacterial taxa, including: class *Bacilli* (β: −0.016, 95% CI −0.029 to −0.004, $P = 0.013$), order *Lactobacillales* (β: −0.017, 95% CI −0.030 to −0.004, $P = 0.01$), family *Lactobacillaceae* (β: −0.024, 95% CI −0.046 to −0.001, $P = 0.037$), family *Streptococcaceae* (β: −0.018, 95% CI −0.031 to −0.004, $P = 0.009$), and genus *Streptococcus* (β: −0.018, 95% CI −0.032 to −0.005, $P = 0.006$). Class *Bacteroidia* and order *Bacteroidales* exhibited identical IV and MR outcomes. We retained the lower bacterial taxonomic level (order *Bacteroidales*) for subsequent analysis. Overall, HIV infection was shown to increase the abundance of three *Bacteroidetes* taxa at the phylum (*Bacteroidetes*), class (*Bacteroidia*) and order (*Bacteroidales*) levels, as well as one *Proteobacteria* bacterial taxon at the genus (*Sutterella*)

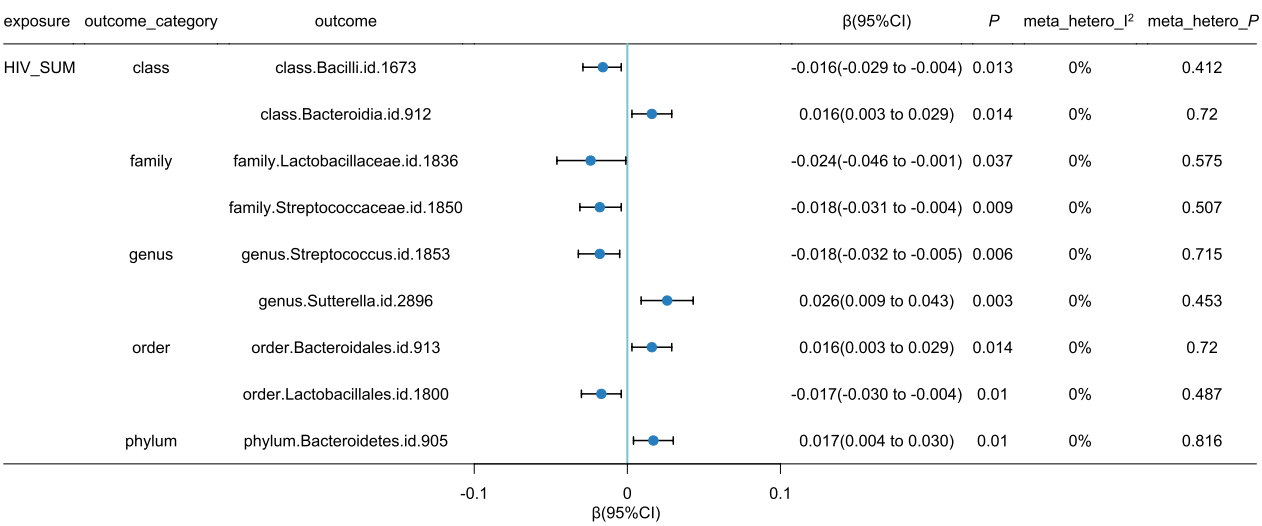

Fig. 2 The meta-analysis combining the primary MR analyses (IVW) of the causal effects of HIV infection on gut microbiota deriving from FinnGen and UK Biobank datasets. CI, confidence intervals; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P* value of Cochran's Q test of meta-analysis

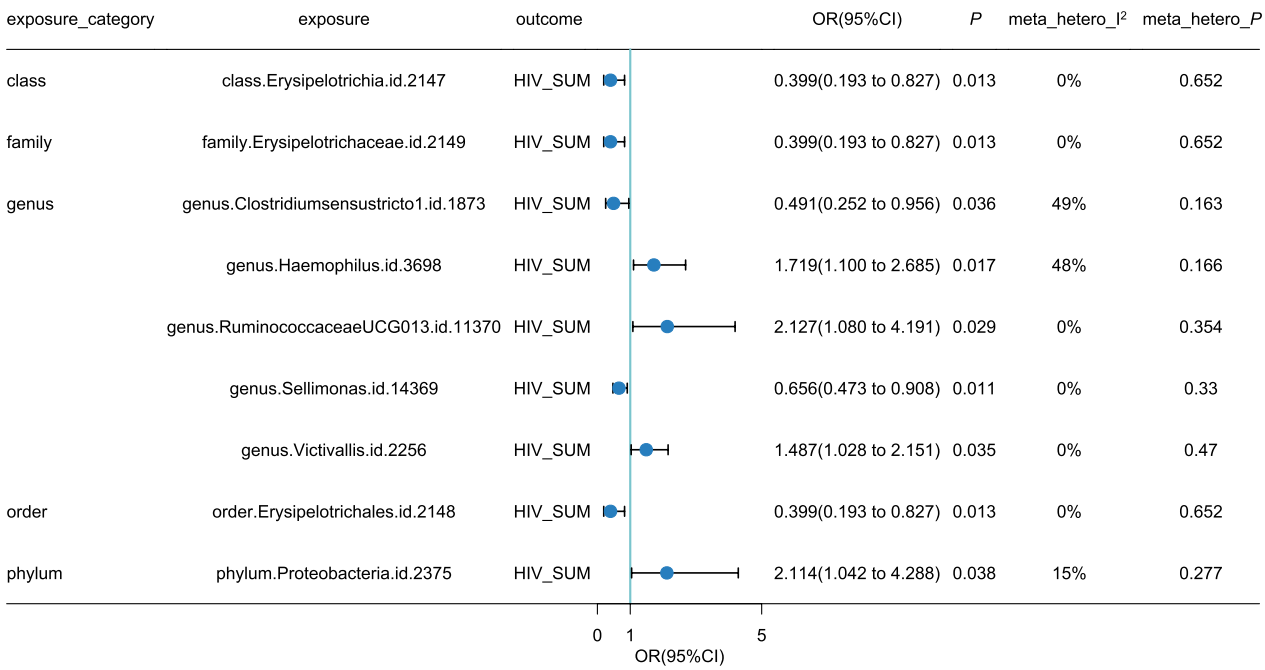

Fig. 3 The meta-analysis combining the primary MR analyses (IVW) of the causal effects of gut microbiota on HIV infection deriving from FinnGen and UK Biobank datasets. CI, confidence intervals; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P* value of Cochran's Q test of meta-analysis; OR, odds ratio

level, while decreasing the abundance of five *Firmicutes* taxa at the class (*Bacilli*), order (*Lactobacillales*), family (*Lactobacillaceae*, *Streptococcaceae*), and genus (*Streptococcus*) levels.

Causal effects of gut microbiota on HIV

Meta-analysis using the IVW method supported the causal effects of nine bacterial taxa on the risk of HIV infection (Fig. 3, Supplementary Figure S2, and Supplementary Tables S4–S6). A higher abundance of two phylum *Proteobacteria* bacterial taxa, specifically:

phylum *Proteobacteria* (odds ratio [OR]: 2.114, 95% CI 1.042–4.288, $P=0.038$), genus *Haemophilus* (OR: 1.719, 95% CI 1.100–2.685, $P=0.017$), as well as two phylum *Firmicutes*, class *Clostridia* bacterial taxa: genus *Ruminococcaceae UCG013* (OR: 2.127, 95% CI 1.080–4.191, $P=0.029$), and genus *Victivallis* (OR: 1.487, 95% CI 1.028–2.151, $P=0.035$) exhibited causal effects on the increased susceptibility of HIV infection. Conversely, a higher abundance of three phylum *Firmicutes*, class *Erysipelotrichia* bacterial taxa: class *Erysipelotrichia* (OR: 0.399, 95% CI 0.193–0.827, $P=0.013$), order *Erysipelotrichales* (OR: 0.399, 95% CI 0.193–0.827, $P=0.013$), family *Erysipelotrichaceae* (OR: 0.399, 95% CI 0.193–0.827, $P=0.013$), as well as two phylum *Firmicutes*, class *Clostridia* bacterial taxa: genus *Clostridium sensu stricto1* (OR: 0.491, 95% CI 0.252–0.956, $P=0.036$), and genus *Sellimonas* (OR: 0.656, 95% CI 0.473–0.908, $P=0.011$) were causally associated with a lower risk of HIV infection. Class *Erysipelotrichia*, order *Erysipelotrichales*, and family *Erysipelotrichaceae* exhibited the same IVs and MR results. Thus, we used the lowest taxa (family *Erysipelotrichaceae*) for the subsequent analysis. Taken together, *Proteobacteria* was shown to increase the risk of HIV infection at the phylum (*Proteobacteria*) and genus (*Haemophilus*) levels, while *Firmicutes* demonstrated both risk-increasing and risk-reducing effects on HIV infection, with class *Erysipelotrichia* reducing the risk at the class (*Erysipelotrichia*), order (*Erysipelotrichales*), and family (*Erysipelotrichaceae*) levels, and class *Clostridia* showing both risk-increasing (*Ruminococcaceae* and *Victivallis*) and risk-reducing (*Clostridium* sensu stricto *1* and *Sellimonas*) effects on HIV infection.

Causal effects of HIV on plasma metabolites

The results from the meta-analysis regarding the causal effects of HIV infection on 53 plasma metabolites are shown in Supplementary Figure S3 and S4, and Supplementary Tables S7–S9. The findings of the combined IVW results revealed that symptomatic HIV infection was causally correlated with the elevated levels of three metabolite ratios and 18 metabolites. Of the 18 plasma metabolites, 13 had known identities across superpathways (i.e., amino acids [n = 7],

vitamins [n = 1], and xenobiotics [n = 5]). The remaining five metabolites were categorized as unknown molecules. The beta of *plasma metabolites* ranged from −0.019 (95% CI −0.038 to −0.000, *P* = 0.05) for Inosine to theophylline ratio to −0.011 for Homoarginine (95% CI −0.022 to −0.000, *P* = 0.045), 1-stearoyl-GPI (18:0) (95% CI −0.022 to 0.000, *P* = 0.047), and X-25420 (95% CI −0.022 to −0.001, *P* = 0.034) levels. HIV infection causally decreased the levels of five metabolite ratios and 27 metabolites. These metabolites were implicated in 22 superpathways, encompassing amino acids (n = 4), lipids (n = 11), nucleotides (n = 2), peptides (n = 1), and xenobiotics (n = 4), in addition to one partially characterized molecule and four unknown metabolites. The beta of plasma metabolites ranged from 0.011 (95% CI 0.000–0.022, *P* = 0.042) for Homostachydrine levels to 0.024 (95% CI 0.009–0.038, *P* = 0.001) for X-24801 levels.

Causal effects of plasma metabolites on HIV

The meta-analyses that combined the main MR (IVW) for the causality of metabolites on HIV are summarized in Supplementary Figure S5 and S6, and Supplementary Tables S10–S12. The levels of 15 metabolites and four metabolite ratios were found to causally increase the risk of HIV infection. These metabolites were implicated in 13 superpathways encompassing amino acids (n = 4), carbohydrates (n = 1), lipids (n = 6), nucleotides (n = 1), and xenobiotics (n = 1), in addition to two unknown metabolites. The OR ranged from 1.165 (95% CI 1.004–1.352), *P* = 0.044) for 5alpha-androstan-3alpha, 17beta-diol monosulfate (1) levels to 2.223 (95% CI 1.120–4.453, *P* = 0.023) for Salicyluric glucuronide levels. The levels of 23 metabolites and four metabolite ratios were found to causally reduce the risk of HIV infection. These metabolites were involved in 20 superpathways of amino acids (n = 4), lipids (n = 11), and xenobiotics (n = 5), as well as three unknown metabolites. The OR ranged from 0.417 (95% CI 0.253–0.688), *P* = 0.001) for Salicylate/citrate ratio to 0.806 (95% CI 0.652–0.996, *P* = 0.046) for Andro steroid monosulfate C19H28O6S (1) levels. Notably, only one bidirectional causal relationship was identified between 1-palmitoyl-GPI (16:0) and HIV infection (Supplementary Figure S3–S6). 1-Palmitoyl-GPI (16:0) is a protective factor for HIV infection (OR: 0.653, 95% CI 0.435–0.980, *P* = 0.04; converted β: −0.426, 95% CI −0.832 to −0.02, *P* = 0.04, Supplementary Figure S5–S6). However, the causal effect of HIV on 1-palmitoyl-GPI (16:0) is quite small (β: −0.012, 95% CI −0.023 to −0.001, *P* = 0.031, Supplementary Figure S3–S4).

Causality between gut microbiota and plasma metabolites

Causal effects of gut microbiota on plasma metabolites

Supplementary Figure S7 and Supplementary Table S13 present the 18 causal relationships of gut microbiota on plasma metabolites. The IVW technique outcomes revealed that family *Erysipelotrichaceae* was suggestively correlated with an elevated level of creatine (β: 0.167, 95% CI 0.001–0.332, *P* = 0.049) and the ratio of 3-methyl-2-oxovalerate to 4-methyl-2-oxopentanoate (β: 0.193, 95% CI 0.012–0.374, *P* = 0.036). Genus *Clostridium sensu stricto 1*causally decreased the levels of 5α-androstan-3α,17β-diol monosulfate (β: −0.159, 95% CI −0.317 to −0.001, *P* = 0.048), and increased the levels of X-25957 (β: 0.174, 95% CI 0.005–0.343, *P* = 0.043). Genus *Haemophilus* demonstrated a causal correlation with the decline of N-acetyl-3-methylhistidine (β: −0.126, 95% CI −0.243 to −0.009, *P* = 0.034). Genus *Ruminococcaceae UCG013* was causally associated with a higher N-acetyl-3-methylhistidine level (β: 0.189, 95% CI 0.007–0.370, *P* = 0.042) and an increased N-acetyl-2-aminoadipate level (β: 0.183, 95% CI 0.022–0.345, *P* = 0.026), as well as an increased level of X-12407 level (β: 0.184, 95% CI 0.013–0.355, *P* = 0.035). Genus *Sellimonas* was causally linked to a higher 5-dodecenoylcarnitine (C12:1) level (β: 0.111, 95% CI 0.031–0.191, *P* = 0.006). Genus *Victivallis* exhibited a causal effect on the elevated levels of 4-hydroxy-2-oxoglutaric acid (β: 0.094, 95% CI 0.015–0.172, *P* = 0.019). Additionally, phylum *Proteobacteria* causally increased the levels of salicyluric glucuronide (β: 0.203, 95% CI 0.003–0.404, *P* = 0.047), N,N,N-trimethyl-5-aminovalerate (β: 0.182, 95% CI 0.023–0.342, *P* = 0.025), 1-palmitoyl-2-oleoyl-gpc (16:0/18:1) (β: 0.186, 95% CI 0.009–0.362, *P* = 0.039), and eicosapentaenoate (EPA; 20:5n3) (β: 0.218, 95% CI 0.061–0.376, *P* = 0.007). In summary, 16 positive causal relationships of gut microbiota on plasma metabolites were identified. Among these, the effect of genus *Sellimonas* on 5-dodecenoylcarnitine (C12:1) was the weakest (β: 0.111), while phylum *Proteobacteria* had the most pronounced effect on eicosapentaenoate (β: 0.218). Additionally, two negative causal relationships were found: genus *Haemophilus* on N-acetyl-3-methylhistidine (β: −0.126) and genus *Clostridium* sensu stricto *1* on 5α-androstan-3α,17β-diol monosulfate (β: −0.159).

Causal effects of plasma metabolites on gut microbiota

The results of IVW analyses revealed the 19 causal effects of plasma metabolites on gut microbiota, as shown in Supplementary Figure S8 and Supplementary Table S14. Andro steroid monosulfate $C_{19}H_{28}O_6S$ (1) and sphingomyelin (d18:2/24:1, d18:1/24:2) were positively associated with the abundance of family *Erysipelotrichaceae* (β: 0.067, 95% CI 0.001–0.134, *P* = 0.048; β: 0.089, 95% CI

0.003–0.175, $P=0.041$). N-acetylthreonine, 1-stearoyl-2-oleoyl-GPE (18:0/18:1), and caprylate (8:0) were causally associated with a higher abundance of genus *Clostridium sensu stricto 1* (β: 0.128, 95% CI 0.016–0.239, $P=0.025$; β: 0.066, 95% CI 0.004–0.128, $P=0.038$; β: 0.146, 95% CI 0.035–0.258, $P=0.01$). In addition, (2 or 3)-decenoate (10:1n7 or n8) and the salicylate to citrate ratio were linked to an increase in genus *Ruminococcaceae UCG013* (β: 0.110, 95% CI 0.011–0.208, $P=0.03$; β: 0.143, 95% CI 0.009–0.278, $P=0.037$). 1-Palmitoyl-GPI (16:0), 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4), and EPA (20:5n3) were negatively associated with the abundance of genus *Haemophilus* (β: −0.268, 95% CI −0.432 to −0.103, $P=0.001$; β: −0.164, 95% CI −0.272 to −0.055; β: −0.169, 95% CI −0.325 to −0.013, $P=0.034$), while 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) was positively linked to the abundance of genus *Haemophilus* (β: 0.139, 95% CI 0.043–0.236, $P=0.005$). 4-Allylphenol sulfate, dimethylglycine and 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) exhibited a negative correlation with the presence of phylum *Proteobacteria* (β: −0.122, 95% CI −0.224 to −0.020, $P=0.019$; β: −0.051, 95% CI −0.102 to −0.001, $P=0.044$; β: −0.067, 95% CI −0.131 to −0.003, $P=0.041$). Conversely, the 3-phosphoglycerate to adenosine 5'-diphosphate (ADP) ratio displayed a positive association with the abundance of phylum *Proteobacteria* (β: 0.106, 95% CI 0.014–0.199, $P=0.025$). Overall, 13 positive causal relationships of plasma metabolites on gut microbiota were identified. Among these, the effect of 1-stearoyl-2-oleoyl-GPE (18:0/18:1) on genus *Clostridium sensu stricto 1* was the weakest (β: 0.066), while Caprylate (8:0) had the most pronounced effect on genus *Clostridium* sensu stricto *1* (β: 0.146). Additionally, six negative causal relationships were found: beta from −0.268 (for 1-palmitoyl-GPI (16:0) on genus *Haemophilus*) to −0.051 (Dimethylglycine on phylum *Proteobacteria*).

Mediation analysis

Using metabolites as a mediator, a mediation relationship displaying statistical significance was established. Genus *Sellimonas* was found to enhance the plasma level of 5-dodecenoylcarnitine (C12:1) (β=0.111, 95% CI 0.031–0.191, $P=0.006$), which, in turn, increased the risk of HIV infection. The proportion of 5-dodecenoylcarnitine (C12:1) mediation was 13.7% (95% CI=0.605–32.882%, $P=0.0348$) (Fig. 4a, Table S16).

Furthermore, we found that genus *Haemophilus* acted as a mediator in three mediation relationships (Fig. 4b–d). 1-Palmitoyl-GPI (16:0) causally decreased the abundance of genus *Haemophilus* (β=−0.268, 95% CI −0.432 to −0.103, $P=0.001$) and was found to be subsequently

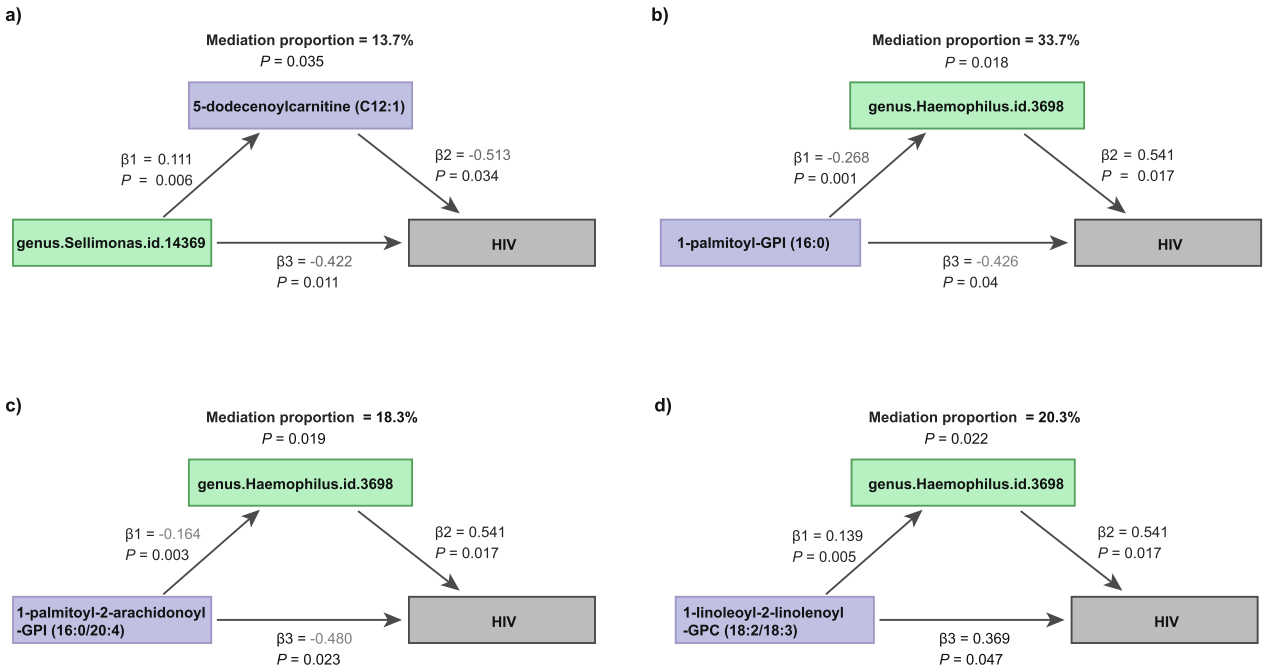

Fig. 4 Mediation analysis. **a** 5-dodecenoylcarnitine (C12:1) mediated the causal effect of *genus Sellimonas* on HIV infection. **b** *Genus Haemophilus* mediated the causal effect of 1-palmitoyl-GPI (16:0) on HIV infection. **c** *Genus Haemophilus* mediated the causal effect of 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4) on HIV infection. **d** *Genus Haemophilus* mediated the causal effect of 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) on HIV infection

associated with a declined risk of HIV infection, with a mediation proportion of 33.7% (95% CI = 4.510–74.59%, $P = 0.018$) (Fig. 4b, Table S15). Likewise, 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4) reduced the abundance of genus *Haemophilus* (β = −0.164, 95% CI −0.272 to −0.055, $P = 0.003$). This decrease was causally associated with a lowered risk of HIV infection, with a mediation proportion of 18.3% (95% CI = 2.162–41.229%, $P = 0.019$) (Fig. 4c, Table S15). Conversely, 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) was found to be causally linked to an elevation in the abundance of genus *Haemophilus* (β = 0.139, 95% CI 0.043–0.236, $P = 0.005$). This increase was associated with a higher risk of HIV infection, with a mediation proportion of 20.3% (95% CI = 2.079–46.320%, $P = 0.0216$) (Fig. 4d, Table S15).

Discussion

Causality between HIV infection and gut microbiota

In healthy individuals, the predominant gut microbiota primarily consists of two main phyla—the *Bacteroidetes* and *Firmicutes*. These two phyla, along with additional phyla, namely the *Proteobacteria, Actinobacteria, Synergistetes,* and *Fusobacteria,* encompass almost all of the bacterial species found in human gut microbiome [39, 40]. This complex assembly of microorganisms plays a crucial role in both host health and disease [41]. Numerous studies have established a correlation between HIV infection and microbiome dysbiosis [2–5, 11, 42–50]. However, due to confounding factors, the results of research show significant variability [5]. MR can eliminate the influence of confounding factors and does not require intervention on participants, affording a practicality unattainable by clinical research studies. In the present study, we first elucidated the causal relationships between symptomatic HIV infection and gut microbiota using MR.

Most of the available data on HIV-related gut dysbiosis indicates an increase in the population of potentially pathogenic phylum *Proteobacteria* and a decrease in that of beneficial commensals such as phyla *Bacteroidetes* and *Firmicutes* [2, 5, 44, 51]. The present study showed that HIV infection could increase the abundance of phylum *Bacteroidetes* (including class *Bacteroidia* and order *Bacteroidales*) and phylum *Proteobacteria* (including genus *Sutterella*) and decrease the abundance of phylum *Firmicutes* (including class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae*, family *Streptococcaceae*, and genus *Streptococcus*). The results regarding phyla *Proteobacteria* and *Firmicutes* were consistent with those of previous studies [2, 5, 44, 51]. Interestingly, alterations in the population of phylum *Bacteroidetes* in individuals with HIV were contrary to that reported in previous reports [2, 5, 51]. This change needs to be further investigated from the perspectives of diversity and composition ratios of gut bacteria.

The majority of current studies are focused on alterations in gut microbiota following HIV infection [2, 3, 11, 42, 44–50]. Only a few studies have shown that pre-existing pathogenic alteration in the gut microbiome were observed in individuals with HIV-1 infection, indicating the microbiome's influence on HIV susceptibility and the risk of developing AIDS [4, 5]. In the present study, we initially focused on the causal effects of HIV on gut microbiota. After converting the OR into β coefficients [β = ln (OR)], the absolute values of the β coefficients (|β|) for the causal effect of gut microbiota on HIV were found to vary from 0.397 to 0.919, whereas those for the causal effect of HIV on gut microbiota ranged from 0.016 to 0.026. Surprisingly, we found that the causal effects of gut microbiota on the risk of HIV infection are considerably more substantial. The MR results show that phylum *Proteobacteria* and genus *Ruminococcaceae UCG013* exhibited a notable adverse causal effect on HIV infection (with an OR exceeding 2), whereas genus *Clostridium sensu stricto 1* and family *Erysipelotrichaceae* acted as fairly significant protective factors for HIV (with an OR below 0.5). Family *Erysipelotrichaceae* has been observed to be significantly elevated prior to HIV-1 infection [4], aligning with our study results. However, the impact of the other three taxa on the risk of HIV infection has not been reported. Our study results enrich the current limited understanding of the role of gut microbiota dysbiosis in the risk and development of HIV infection.

Causality between HIV infection and plasma metabolites

The results of MR analyses reveal the causal effects of HIV infection on 53 plasma metabolites. The absolute values of β coefficients (|β|) ranged from 0.011 to 0.024. It is worth noting that |β| for the causal effect of 46 plasma metabolites on HIV infection ranged from 0.153 to 0.875 (after converting the OR into β coefficients). Therefore, the causal effects of plasma metabolites on the risk of HIV infection were more substantial than the impact of HIV infection on metabolites. Meta-analyses conducted using the IVW method showed that the salicyluric glucuronide level exhibited a considerably adverse causal effect on HIV infection with an OR exceeding 2. Conversely, the salicylate-to-citrate ratio was identified as a significant protective factor for HIV with an OR of < 0.5.

Aspirin (acetylsalicylic acid) is a famous drug containing salicylic acid. Salicyluric glucuronide is one of the major metabolites that salicylic acid produces [52]. No convincing evidence is yet available to show that aspirin can affect the risk of HIV infection. There is only one study indicating that low-dose aspirin in HIV-uninfected women may reduce T-cell activities, thus potentially

preventing HIV infection [53]. The results of this study suggested that aspirin could be associated with the risk of HIV infection; however, we could not determine whether aspirin was an adverse or a protective factor. Salicylate and citrate share the same transporter using metabolite–protein associations recorded in the Human Metabolome Database (HMDB) [21]. Therefore, the salicylate-to-citrate ratio was calculated in the original research [21]. Citrate plays a significant role in energy metabolism. In the tricarboxylic acid cycle, citrate is metabolized into various metabolic products, releasing energy [54]. Therefore, we hypothesized that energy metabolism influences the metabolic process of salicylic acid, affecting the risk of HIV infection. The role of aspirin and its metabolite composition in HIV infection risk needs to be rigorously investigated for validation.

Only one bidirectional causal relationship has been identified between 1-palmitoyl-GPI (16:0) and HIV infection. 1-Palmitoyl-GPI, also known as 1-hexadecanoyl-sn-glycero-3-phospho-(1'-myo-inositol), is a specific form of glycerophosphoinositol molecules [55]. There have been no studies specifically reporting the correlation between 1-palmitoyl-GPI and HIV. Previous studies have reported that individuals who eventually acquire HIV have slightly higher levels of glycerophosphoinositol compared to matched controls prior to HIV infection [5]. This suggests that glycerophosphoinositol might play a role in the susceptibility or early process of HIV infection. Our findings, however, indicate that 1-palmitoyl-GPI is a protective factor. The discrepancy in these research results may be due to glycerophosphoinositols encompassing various molecular forms within a broader classification framework, some of which may have detrimental effects in HIV infection. Further research is needed to elucidate the role of glycerophosphoinositols in HIV infection.

Mediation relationship

Genus *Sellimonas* was found to enhance the plasma level of 5-dodecenoylcarnitine (C12:1), increasing the risk of HIV infection. 5-Dodecenoylcarnitine (C12:1) is involved in fatty acid metabolism [21]. Therefore, Genus *Sellimonas* may reduce the risk of HIV infection via lipid metabolism.

Genus *Haemophilus* acts as a mediator in three mediation relationships involving phospholipids. Mediation analysis indicated that 1-palmitoyl-GPI (16:0) and 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4) exhibit protective effects on the risk of HIV infection mediated by a reduction in the population of genus *Haemophilus*. Conversely, 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) is causally associated with a higher risk of HIV infection owing to an elevation in the abundance of *Haemophilus*. 1-palmitoyl-GPI (16:0) and 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4) are of the phosphatidylinositols (PIs) class; 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) is of the phosphatidylcholines (PCs) class. PIs and PCs are two major classes of glycerophospholipids (GPLs) that play significant roles in cell-membrane construction and signaling pathways [56]. In general, PCs make up 40–50% of the total phospholipids as a major constituent of the plasma membrane (PM) [57]. PIs account for 10% of the total phospholipids and are distributed broadly across most subcellular organelles, but lack at the PM [58]. *Haemophilus influenzae*, the most famous pathogen of the genus *Haemophilus*, is an opportunistic bacterial pathogen. [59]. However, when certain factors (such as viral infections and a weakened immune response) are present, *Haemophilus influenzae* can cause severe infections [60]. Its polysaccharide capsule and lipo-oligosaccharides are important virulence factors that contribute to resistance against complement-mediated phagocytosis [60], which is closely related to membrane phospholipid dynamics and signaling [61]. Our results indicated that the metabolism and function of certain types of cell-membrane phospholipids may alter the abundance of the *Haemophilus* genus, thereby influencing susceptibility to HIV infection.

Conclusion

Our study revealed that gut microbiota and metabolites exert causal influence on HIV infection risk more substantially than the reverse. We identified one bidirectional causality between 1-palmitoyl-GPI (16:0) and HIV infection, as well as four mediation relationships. Genus Haemophilus mediated the causal effects of 1-palmitoyl-GPI (16:0), 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4), and 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) on HIV-infection risk. Additionally, 5-Dodecenoylcarnitine (C12:1) mediated the causal effect of genus Sellimonas on the risk of HIV infection. These findings contribute to a deeper understanding of the pathophysiology of HIV infection and provide novel insights for primary prediction, targeted prevention, and personalized treatment of AIDS.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-024-02480-1.

Additional file 1: Figure S1. Forest plot illustrating the causal effects of HIV infection on gut microbiota (including MR results from the FinnGen and UK Biobank datasets, and a meta-analysis of both above). CI, confidence intervals; hetero_*P*, *P*-value of Cochrane Q test assessing heterogeneity of MR analysis; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P*-value of Cochran's Q test of meta-analysis; presso_*P*, *P*-value of MR-PRESSO evaluating horizontal pleiotropy effect of MR results; nsnp, the number of SNPs; *P*, *P*-value of MR analysis (IVW)

or the meta-analysis; pleio_*P*, *P*-value of MR-Egger regression examining horizontal pleiotropy effect of MR results.

Additional file 2: Figure S2. Forest plot illustrating the causal effects of gut microbiota on HIV infection (including MR results from the FinnGen and UK Biobank datasets, and a meta-analysis of both above). CI, confidence intervals; hetero_*P*, p-value of Cochrane Q test assessing heterogeneity of MR analysis; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P*-value of Cochran's Q test of meta-analysis; presso_*P*, *P*-value of MR-PRESSO evaluating horizontal pleiotropy effect of MR results; nsnp, the number of SNPs; OR, odds ratio; *P*, *P*-value of MR analysis (IVW) or the meta-analysis; pleio_*P*, *P*-value of MR-Egger regression examining horizontal pleiotropy effect of MR results.

Additional file 3: Figure S3. The meta-analysis combining the primary MR analyses (IVW) of the causal effects of HIV infection on plasma metabolites deriving from FinnGen and UK Biobank datasets. CI, confidence intervals; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P*-value of Cochran's Q test of meta-analysis.

Additional file 4: Figure S4. Forest plot illustrating the causal effects of HIV infection on plasma metabolites (including MR results from the FinnGen and UK Biobank datasets, and a meta-analysis of both above). CI, confidence intervals; hetero_*P*, *P*-value of Cochrane Q test assessing heterogeneity of MR analysis; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P*-value of Cochran's Q test of meta-analysis; presso_*P*, *P*-value of MR-PRESSO evaluating horizontal pleiotropy effect of MR results; nsnp, the number of SNPs; *P*, *P*-value of MR analysis (IVW) or the meta-analysis; pleio_*P*, *P*-value of MR-Egger regression examining horizontal pleiotropy effect of MR results.

Additional file 5: Figure S5. The meta-analysis combining the primary MR analyses (IVW) of the causal effects of plasma metabolites on HIV infection deriving from FinnGen and UK Biobank datasets. CI, confidence intervals; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P*-value of Cochran's Q test of meta-analysis; *P*, *P*-value of meta-analysis; OR, odds ratio.

Additional file 6: Figure S6. Forest plot illustrating the causal effects of plasma metabolites on HIV infection (including MR results from the FinnGen and UK Biobank datasets, and a meta-analysis of both above). CI, confidence intervals; hetero_*P*, *P*-value of Cochrane Q test assessing heterogeneity of MR analysis; meta_hetero_I^2, I^2 statistic assessing the heterogeneity of meta-analysis; meta_hetero_*P*, *P*-value of Cochran's Q test of meta-analysis; presso_*P*, *P*-value of MR-PRESSO evaluating horizontal pleiotropy effect of MR results; nsnp, the number of SNPs; OR, odds ratio; *P*, *P*-value of MR analysis (IVW) or the meta-analysis; pleio_*P*, *P*-value of MR-Egger regression examining horizontal pleiotropy effect of MR results.

Additional file 7: Figure S7. Forest plot illustrating the causal effects of gut microbiota on plasma metabolites using IVW method. CI, confidence intervals; hetero_*P*, *P*-value of Cochrane Q test assessing heterogeneity of MR analysis; presso_*P*, *P*-value of MR-PRESSO evaluating horizontal pleiotropy effect of MR results; *P*, *P*-value of MR analysis using IVW method; pleio_*P*, *P*-value of MR-Egger regression examining horizontal pleiotropy effect of MR results.

Additional file 8: Figure S8. Forest plot illustrating the causal effects of plasma metabolites on gut microbiota using IVW method. CI, confidence intervals; hetero_*P*, *P*-value of Cochrane Q test assessing heterogeneity of MR analysis; presso_*P*, *P*-value of MR-PRESSO evaluating horizontal pleiotropy effect of MR results; *P*, *P*-value of MR analysis using IVW method; pleio_*P*, *P*-value of MR-Egger regression examining horizontal pleiotropy effect of MR results.

Additional file 9: Supplementary Table S1-S16.

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Author contributions
JH: data curation; funding acquisition; formal analysis; writing—review and editing. JH: writing—original draft preparation; writing—review and editing. DH: conceptualization; data curation; formal analysis; methodology; project administration; writing—review and editing.

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Availability of data and materials
The GWAS summary statistics used in this study are publicly available through the international consortium MiBioGen (https://mibiogen.gcc.rug.nl/menu/main/home), the Canadian Longitudinal Study on Aging (CLSA) cohort (https://www.ebi.ac.uk/gwas/publications/36635386), FinnGen consortium R7 release data (https://r7.risteys.finngen.fi/phenocode/AB1_HIV) and the UK Biobank (UKB) data (https://www.ebi.ac.uk/gwas/studies/GCST90041717). All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Ethical approval and participant consent were obtained in the original studies (MiBioGen, CLSA, FinnGen and UKB).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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