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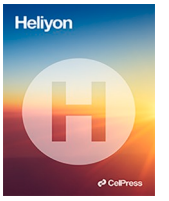

Research article

Interleukin-2 mediated associations between gut microbiota and acute myeloid leukemia: A population-based mediation Mendelian randomization study

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ABSTRACT

The relationship between the gut microbiota and acute myeloid leukemia (AML) has been established, but the exact role of interleukin (IL) in mediating this relationship has remained unclear. This study aimed to utilize whether interleukins mediate the relationships between gut microbiota and AML, thereby identifying potential novel targets for future AML treatment. Mendelian randomization (MR) is a method for finding the causality of exposure and outcome. Final instrumental variables were selected based on MR assumptions, and used to judge validity of the results. Our study identified risk and protective factors for AML, and interleukin-related gut microbiota. Finally, mediation MR analyses resulted in Interleukin-2 (IL-2) mediated associations between *Clostridiaceae 1*, *Clostridium sensu stricto 1* and AML, with IL-2 respectively explaining 13.96 % and 12.11 % of the total effect of the aforementioned gut microbiota on AML. Our results successfully identified causal effects between specific gut microbiota, AML, and interleukins, while also elucidating the mediating role of IL-2 in these associations using MR analysis. These findings provide valuable insights into potential therapeutic targets for AML treatment.

1. Introduction

Acute myeloid leukemia (AML) is the most prevalent acute leukemia in adults, with its incidence escalating notably with advancing age, with the median age at diagnosis usually ranging from 65 to 72 years in Western countries [1]. A registry study reported that the world age-standardized incidence rate of leukemia is 46.4 per million per year in children aged 0–14 years and 28.5 per million in adolescents aged 15–19 years [2]. In the United States, approximately 20,050 individuals across all age groups were diagnosed with AML in 2022, resulting in 11,540 deaths attributable to AML [3]. Ionizing radiation and occupational exposure to benzene and petrochemicals are associated with AML. Previous study has shown that exposure to radiation therapy and nuclear facilities may increase the risk of AML [4]. Furthermore, benzene derivatives cause chromosomal abnormalities and epigenetic changes, further increasing the risk of AML [5]. Despite advancements in AML treatment understanding in recent years, conventional treatment modalities may not suffice for refractory/relapsed cases [6]. For patients deemed unsuitable for intensive chemotherapy, treatment with azacitidine (AZA) or decitabine plus venetoclax (VEN) has become the standard of treatment [7]. However, a cohort study reported

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that only 12 % of refractory/relapsed patients exhibited a response, with a median overall survival (OS) of 3.1 months after the AZA and VEN treatment [8]. Considering the limitations of current treatment methods, it was critical to explore the potential new treatment target of AML.

The gut microbiota and its metabolites have been recognized for their significant roles in the immune and hematologic systems [9,10]. For instance, a microbiota-derived polysaccharide has been demonstrated to induce an anti-inflammatory gene signature in murine intestinal macrophages [11]. Several observational studies and mice experiments investigated the association between the gut microbiota and AML. A study revealed that the diversity and composition of the gut microbiota were significantly altered in AML patients, and the gut microbiota dysbiosis further aggravated the progression of AML [12]. Recent research have highlighted a correlation between higher gut microbiota diversity and reduced infection risk during induction chemotherapy in AML patients [13,14]. A multicenter clinical trial involving 62 AML patients reported a decrease in antibiotic resistance genes and an increase in gut microbiota diversity in AML patients following autologous fecal microbiota transfer (AFMT) [15]. The validity of AFMT underscores the therapeutic potential of gut microbiota manipulation as an option to restore diversity and serve as a promising therapeutic target for AML management [15]. The impact of the gut microbiota on AML progression may be mediated through its modulation of host metabolism or immune response [10]. However, the potential mechanism by which gut microbiota regulates AML progression has not been fully explored.

Relationships between gut microbiota and interleukins were pointed out by previous studies [16,17]. Yang et al. reported that gut microbiota induced IL-22 production by modulating short-chain fatty acids (SCFAs) levels [16]. Similarly, Fang et al. found the gut microbial genus *Clostridium sensu stricto 1* was positively correlated with IL-1β [17]. In the meanwhile, interactions between interleukins and AML also were demonstrated by previous research. Molica et al. revealed that IL-2 can be used in immunotherapy against AML [18]. Additionally, studies have shown that IL-34-based differentiation strategies and IL-4 treatment were promising strategies to treat AML [19–21]. Therefore, we assume that interleukin may play a mediating role in gut microbiota and AML based on previous findings.

Mendelian randomization (MR) is a method that utilizes the principles of Mendelian heredity to test for causal relationships between exposures and outcomes, thus minimizing the impact of confounding factors (such as lifestyle factors) [22]. A previous Mendelian randomization study investigated that higher levels of IL-18 were associated with a decreased risk of AML [23]. Therefore, to elucidate the potential effects of gut microbiota-associated interleukins on AML, we conducted the mediation MR analysis using public genome-wide association studies (GWAS) data.

2. Methods

2.1. Characteristics of cohorts

2.1.1. Gut microbiota

For gut microbiota traits, 18,340 participants from 24 cohorts were included for 16S rRNA gene sequence analysis in the original study [24]. Participants were enrolled mainly from Europe countries. For each cohort, taxa present in more than 10 % of samples were included in the quantitative microbiota trait loci (mbQTL) mapping. After mbQTL mapping, 211 taxa (9 phyla, 16 classes, 20 orders, 35 families, and 131 genera) were involved in mbQTL analysis. Ethical approval and consent were confirmed for all of the individuals in cohorts. Summary levels of mbQTL analysis results were available at the www.mibiogen.org website.

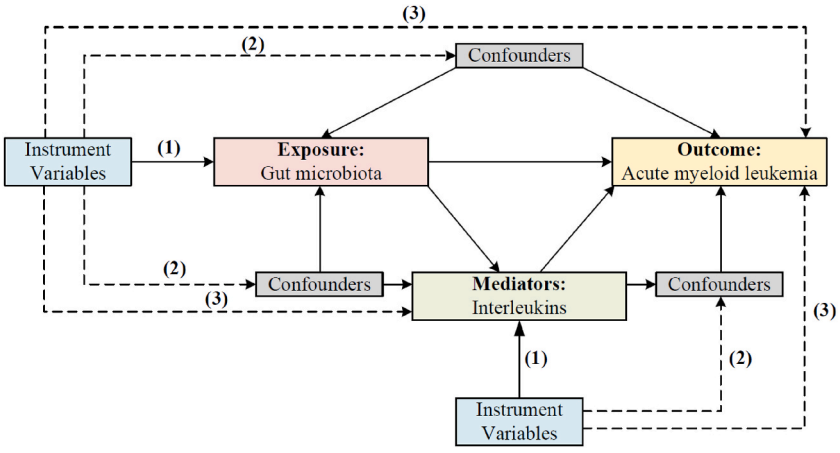

Fig. 1. The flowchart of current MR study. Note: This study based on three assumptions: (1) the IVs should be significantly connected to the exposure; (2) the IVs should not be connected to any known confounders that could alter the association between an exposure and an outcome; and (3) the IVs should be unrelated to the outcomes and may only affect the outcomes through their effects on the exposure. This figure illustrates a diagram of current MR study. The dash lines indicate irrelevance, and the solid lines indicate relevance.

2.1.2. Interleukins

We selected IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-17A, and IL-21 in present analyses. Interleukins were measured through plasma protein levels from the INTERVAL study [25]. After the process of genetic quality control, 3301 individuals (2481 and 820) from two non-overlapping sub-cohorts remained for analysis. All participants acquired informed consent and the National Research Ethics Service approved this study (11/EE/0538).

2.1.3. Acute myeloid leukemia

We acquired summary-level data of AML (GCST90042758) from publicly available genome-wide association study (GWAS) studies. We obtained summary statistics available data from an open website at https://www.ebi.ac.uk/gwas/. All 456,348 participants of cohorts of AML were European. The original study was granted ethical approval and informed consent of the participants.

2.2. Statistical analysis

2.2.1. Two-step MR analysis method

In the present study, we conducted a two-step mediation Mendelian randomization (MR) analysis to investigate the potential role of interleukins in mediating the associations between gut microbiota and AML (Fig. 1). Firstly, we examined the causal effects of gut microbiota on AML to identify specific taxa that are associated with AML. Additionally, we assessed the causal effects of these specific taxa on interleukin levels. Secondly, taxa significantly associated with both interleukin and AML were included for multivariable MR analysis. The total effect of gut microbiota was then decomposed into a direct effect (i.e., the effect of gut microbiota on AML independent of the mediators) and an indirect effect (i.e., the effect of gut microbiota on AML via the mediator). This approach is currently being widely applied in the medical field [26]. Different from traditional observational mediation analysis approaches, this approach was both sensitive to the causal effects of the mediator and corrected for its measurement error [26]. Further analyses were based on the above theories.

2.2.2. Instrument variables selection

In two-sample MR and mediation MR analysis, IVs were obtained according to the assumptions of MR. We used R (version 4.3.0) packages of TwoSampleMR (version 0.5.6) and MendelianRandomization (version 0.7.0) for IVs selection in two-sample MR analysis. We selected SNPs that were associated with gut microbiota at a significance level of $p\text{-}value < 1 \times 10^{-5}$. SNPs with linkage disequilibrium ($r^2 = 0.001$, kb $= 10000$) were excluded. SNPs associated with confounding factors ($p\text{-}value < 1 \times 10^{-5}$) that related to gut microbiota and AML were excluded. SNPs that were directly associated with AML ($p\text{-}value < 1 \times 10^{-5}$) were excluded to obtain the IVs. Finally, we calculated the *F*-statistics of IVs to determine the weak IV bias. IVs with weak bias (*F*-statistics <10) were excluded. For finding the causal effect of gut microbiota and interleukins, the selection process of IVs was the same as above.

We used MVMR (version 0.4) for IV selection in multivariable MR analysis [27]. Firstly, IVs associated with gut microbiota ($p\text{-}value < 1 \times 10^{-5}$) were used to evaluate the causal effects of gut microbiota on interleukin. Secondly, IVs associated with interleukin ($p\text{-}value < 1 \times 10^{-5}$) and independent of those used for step one were used to evaluate the effect of interleukin on AML. The thresholds of r^2, kb, and *F*-statistics were consistent with two-sample MR.

2.2.3. Causal effects of gut microbiota and AML, interleukins

We used five MR statistical methods to determine the causal effect between the gut microbiota and AML, interleukins, including inverse-variance weighting (IVW), weighted median, weighted mode, MR-Egger, and simple mode. The Wald ratio was used when only one IV was associated with the exposure to detect the cause-effect. We presented the cause effects of gut microbiota and AML using odds ratios (OR), gut microbiota, and interleukins using beta (β). In the sensitivity analysis, we used Cochran's *Q* test to examine for heterogeneity. We used the MRPRESSO package (version 1.0) to test horizontal pleiotropy using the MR-pleiotropy residual sum and outlier (MR-PRESSO) method [28]. We judged the validity of the five causal effect methods according to the results of sensitivity analysis.

2.2.4. Mediation MR analysis

According to the two-sample results, we selected gut microbiota that were both correlated with interleukins and AML for further MVMR analysis. We used regression-based MVMR to estimate the effect of each mediator on AML. The product of the effect of gut microbiota on interleukin (β1) and the effect of interleukins on AML (β2) was used to estimate the mediating effect (β1*β2) of each interleukin. The proportional mediating effect was obtained by dividing the indirect effect (β1*β2) by the total effect (β). The Delta method and effect estimates were obtained from a two-step MR analysis to derive standard errors [29]. For horizontal pleiotropy testing, we estimate heterogeneity from pleiotropy through *Q*-statistic minimization and removing results with $p\text{-}value < 0.05$.

3. Results

3.1. Causal effects of gut microbiota on AML

Our MR analysis utilized a sample size of 18,340 participants for gut microbiota analysis and 3301 participants for interleukin analysis. Following the two-step multinomial MR analysis, we identified 298 instrumental variables (IVs) to investigate the potential effects of gut microbiota-associated interleukins on AML. These IVs were then subjected to MR analysis to assess their effects.

Ultimately, we retained 9 single nucleotide polymorphisms (SNPs) as our final instrumental variables. (Table 1).

For our main results, we employed the inverse variance weighted (IVW) and Wald ratio methods. Following the IVW analyses, we found that two families of gut microbiota, namely Family *Clostridiaceae 1* and *Family XIII*, exhibited a causal effect on AML. Using the Wald ratio approach, we identified one phylum and one family with three genera that showed causal effects on AML. Specifically, the phylum *Verrucomicrobia*, the family *Alcaligenaceae*, and the genera *Clostridium sensu stricto 1*, *Ruminococcaceae UCG 004*, and *Sutterella* were found to have causal effects on AML. Among these gut microbiota, Family *Alcaligenaceae* (OR = 0.01; 95%CI: 0.00 to 0.21), Genera *Ruminococcaceae UCG 004* (OR = 0.05; 95%CI: 0.01 to 0.39) and *Sutterella* (OR = 0.01; 95%CI: 0.00 to 0.21) were identified as protective factors against AML. Additionally, Family *Clostridiaceae 1* (OR = 10.21; 95%CI: 1.28 to 81.27), Family *FamilyXIII* (OR = 10.68; 95%CI: 1.27 to 89.68), genus *Clostridium sensu stricto 1* (OR = 23.09; 95%CI: 1.36 to 390.65), phylum *Verrucomicrobia* (OR = 18.32; 95%CI: 1.26 to 266.14) were identified as risk factors for AML (Table 2). We also presented the correlations of Family *Clostridiaceae 1* (Fig. 2A), *Family XIII* (Fig. 2B) (nsnp>2), and AML using the IVW method.

3.2. Causal effects of gut microbiota on mediators (interleukins)

To explore the causal effects of the aforementioned gut microbiota on interleukins, we employed a similar strategy. The included IVs are presented in Table 3. Specifically, we focused on the phylum *Verrucomicrobia*, Family *Alcaligenaceae*, Family *Clostridiaceae 1*, *Family XIII*, and the genera *Clostridium sensu stricto 1*, *Ruminococcaceae UCG 004*, and *Sutterella*.

Using either the IVW or Wald ratio methods, we observed that Family *Clostridiaceae 1* (OR = 0.47; 95%CI: 0.24, 0.89) and genus *Clostridium sensu stricto 1* (OR = 0.38; 95%CI: 0.16, 0.92) were negatively associated with IL-2. On the other hand, Family *Alcaligenaceae* (OR = 4.29; 95%CI: 1.84, 10.01) and genus *Sutterella* (OR = 3.90; 95%CI: 1.71, 8.91) were positively associated with IL-9, as determined by the Wald ratio method. Family *Family XIII* showed a positive association with IL-8 (OR = 2.11; 95%CI: 1.10, 4.05), while genus *Ruminococcaceae UCG 004* (OR = 0.42; 95%CI: 0.23, 0.79) and phylum *Verrucomicrobia* (OR = 0.33; 95%CI: 0.14, 0.74) exhibited negative associations with IL-17A and IL-21, respectively (Table 4). Additionally, we presented the correlations between Family *Clostridiaceae 1* (Fig. 3A and B), *Family XIII* (Fig. 3C) (with nsnp>2), and interleukins using the IVW method.

3.3. Mediating role of interleukins in associations between gut microbiota and AML

Then we conducted mediation MR analyses on the associated gut microbiota-interleukin pairs. Our findings revealed a significant mediating role of IL-2 in the associations between AML and Family *Clostridiaceae 1*, as well as Genus *Clostridium sensu stricto 1*. Specifically, our study demonstrated that IL-2 mediated the associations between *Clostridiaceae 1* (OR = 10.21; 95%CI: 1.28 to 81.26) and *Clostridium sensu stricto 1* (OR = 23.09; 95%CI: 1.36 to 390.65) with AML. Furthermore, the Q-statistic, which measures pleiotropy, was greater than 0.05 in both models, indicating the absence of horizontal pleiotropy in these analyses.

The individual proportion effects of mediators are as follows, IL-2 explained 13.96 % (95 % CI:5.21 %, 22.72 %) of the total effect of Family *Clostridiaceae 1* on AML (Fig. 4A), and IL-2 explained 12.11 % (95 %: CI 2.75 %, 21.48 %) of the total effect of genus *Clostridium sensu stricto 1* on AML (Fig. 4B).

4. Discussion

We conducted a two-step multinomial mediation MR analysis to investigate the potential effects of gut microbiota-associated interleukins in AML. We observed significant correlations among gut microbiota, AML, and interleukins Regarding the gut microbiota in patients with AML, we found family *Alcaligenaceae*, genera *Ruminococcaceae UCG 004* and *Sutterella* were protective factors against AML while phylum Verrucomicrobia, family *FamilyXIII*, *Clostridiaceae 1*, and genus *Clostridium sensu stricto 1* were risk factors. As the largest microorganism communities in human bodies, gut microbiota regulate metabolism and immune functions through several pathways [30–32]. For instance, gut microbiota and its metabolites could affect host metabolism by modulating levels of ghrelin, neurotransmitters, and cytokines [30]. Gut microbiota may also affect the immune system by mediating neutrophil migration, which subsequently impacts T-cell differentiation into various types [31]. Additionally, gut microbiota-derived heterogeneous

Table 1
Final IVs included for estimating causal effects of gut microbiota on AML.

Gut microbiota (Exposure)	nSNP	SNP
f_Alcaligenaceae	1	rs9537886
f_Clostridiaceae 1	2	rs2817172
		rs881532
f_Family XIII	2	rs12643275
		rs6501525
g_Clostridium sensu stricto 1	1	rs2817172
g_Ruminococcaceae UCG 004	1	rs6769553
g_Sutterella	1	rs2321387
p_Verrucomicrobia	1	rs12908520

Note: Instrumental variables (IVs) were selected basing the three assumptions of MR study. Final IVs listed in Table 1 were used to estimate the causal effects of gut microbiota on AML.

Table 2
Causal effects of the gut microbiota on AML.

Gut microbiota (Exposure)	Method	nSNP	OR (95%CI)	*p-value*
f_Alcaligenaceae	Wald ratio	1	0.01 (0.00, 0.21)	**<0.01**
f_Clostridiaceae 1	IVW	2	10.21 (1.28, 81.27)	**0.03**
f_Family XIII	IVW	2	10.68 (1.27, 89.68)	**0.03**
g_Clostridium sensu stricto 1	Wald ratio	1	23.09 (1.36, 390.65)	**0.03**
g_Ruminococcaceae UCG 004	Wald ratio	1	0.05 (0.01, 0.39)	**<0.01**
g_Sutterella	Wald ratio	1	0.01 (0.00, 0.21)	**<0.01**
p_Verrucomicrobia	Wald ratio	1	18.32 (1.26, 266.14)	**0.03**

Note: Inverse-variance weighting (IVW) or Wald ratio method was used to estimate the effect of gut microbiota on AML. The effect of gut microbiota on AML was presented as odds ratio (OR) and corresponding 95 % confidence interval (CI).

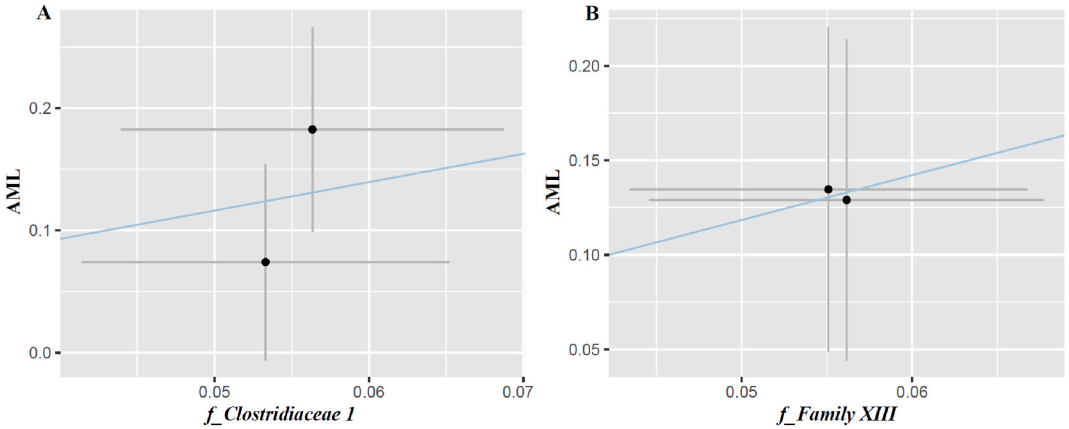

Fig. 2. Scatter plot indicated the effect of gut microbiota on AML. Note: The effect of *f_Clostridiaceae 1* (A) and *f_Family XIII* (B) on AML was calculated through single nucleotide polymorphisms, which provide an association between the gut microbiota and AML through inverse-variance weighting (IVW) method.

Table 3
Final IVs included for estimating causal effects of gut microbiota on IL.

Gut microbiota (Exposure)	nSNP	SNP
f_Alcaligenaceae	1	rs9537886
f_Clostridiaceae 1	2	rs2817172
		rs881532
f_Family XIII	2	rs12643275
		rs6501525
g_Clostridiumsensustricto 1	1	rs2817172
g_Ruminococcaceae UCG 004	1	rs6769553
g_Sutterella	1	rs2321387
p_Verrucomicrobia	1	rs12908520

Note: Instrumental variables (IVs) were selected basing the three assumptions of MR study. Final IVs listed in Table 3 were used to estimate the causal effects of gut microbiota on IL.

molecules may also induce immune response and stimulate inflammation or chronic tissue damage [32]. A recent study reported that gavage with butyrate or *Faecalibacterium* postponed murine AML progression, which indicated that the gut microbiota and its metabolite play a role in AML progression [12]. Gut microbiota have been reported to have either pro- or anti-tumor effects, and regulate not only mucosal but also systemic immune responses [33]. Previous studies demonstrated that the gut microbiota of cancer patients had interaction effects with chemotherapy drugs [34–36]. For instance, *Bifidobacterium* species reduce the adverse effects of chemotherapy by inhibiting proinflammatory cytokines [34]. A cohort study on AML patients revealed significant decreasing in the relative abundances of *Bacteroides*, *Faecalibacterium*, and *Alistipes* during the initial hospitalization, and increased after discharge to levels that were not different from the original period [37]. Additionally, a recent panel study on AFMT in AML patients demonstrated the reconstruction of gut microbiota after AFMT, providing evidence for the effectiveness of AFMT [15]. These findings highlight the dynamic nature of the gut microbiota in the context of AML and suggest the therapeutic potential of targeting the gut microbiota or AML.

A review article has mentioned the correlations between gut microbiota and interleukins, which have garnered significant attention

Table 4
Causal effects of the gut microbiota on IL.

Gut microbiota (Exposure)	Method	IL	nSNP	OR (95%CI)	*p-value*
f_Alcaligenaceae	Wald ratio	IL-9	1	4.29 (1.84, 10.01)	**<0.01**
f_Clostridiaceae 1	IVW	IL-2	2	0.47 (0.24, 0.89)	**0.02**
f_Clostridiaceae 1	IVW	IL-21	2	0.47 (0.25, 0.90)	**0.02**
f_Family XIII	IVW	IL-8	2	2.11 (1.10, 4.05)	**0.03**
g_Clostridium sensu stricto 1	Wald ratio	IL-2	1	0.38 (0.16, 0.92)	**0.03**
g_Ruminococcaceae UCG 004	Wald ratio	IL-17A	1	0.42 (0.23, 0.79)	**<0.01**
g_Sutterella	Wald ratio	IL-9	1	3.90 (1.71, 8.91)	**<0.01**
p_Verrucomicrobia	Wald ratio	IL-21	1	0.33 (0.14, 0.74)	**<0.01**

Note: Inverse-variance weighting (IVW) or Wald ratio method was used to estimate the effect of gut microbiota on IL.
The effect of gut microbiota on IL was presented as odds ratio (OR) and corresponding 95 % confidence interval (CI).

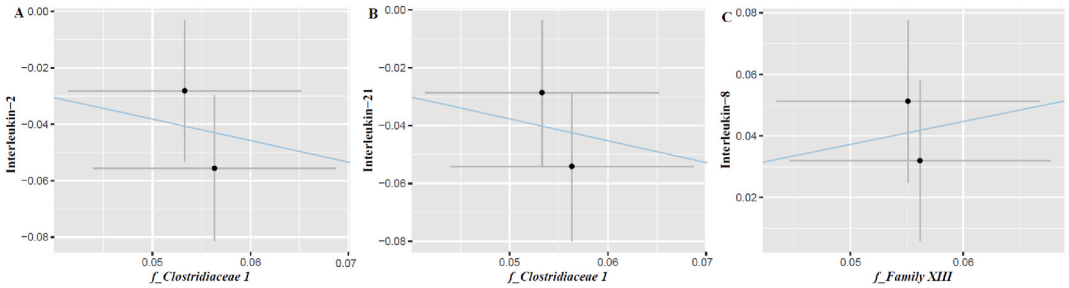

Fig. 3. Scatter plot indicated the effect of gut microbiota on IL. Note: A represented the effect of *f_Clostridiaceae 1* on Interleukin-2; B represented the effect of *f_Clostridiaceae 1* on Interleukin-21; C represented the effect of *f_Family XIII* on Interleukin-8. The effect of the gut microbiota on IL is calculated through single nucleotide polymorphisms, which provide an association between the gut microbiota and IL through inverse-variance weighting (IVW) method.

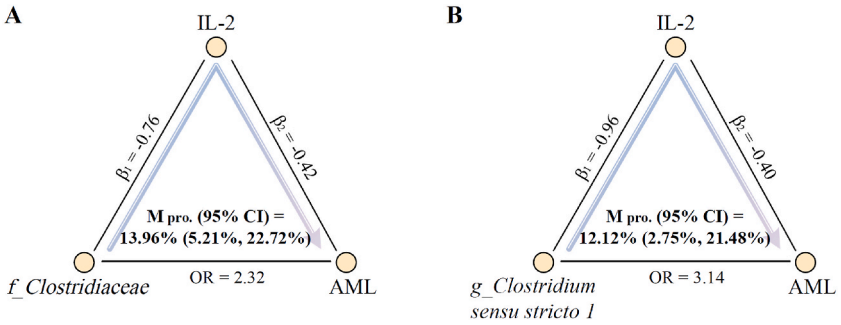

Fig. 4. IL-2 mediated associations between gut microbiota and AML. Note: M pro. indicated mediation proportion. A represented IL-2 could explain 13.96 % (95 % CI: 5.21 %–22.72 %) of association between *f_Clostridiaceae* and AML; B represented IL-2 could explain 12.12 % (95 % CI: 2.75 %–21.48 %) of association between *g_Clostridium sensu stricto 1* and AML.

[38]. In our study, we specifically examined the association between gut microbiota and interleukins such as IL-2, IL-17A, and IL-22. IL-2, a pleiotropic cytokine, plays a crucial role in preventing chronic inflammation within the gastrointestinal tract [39,40]. Notably, low-dose IL-2 immunotherapy could increase TReg cells and prevent chronic inflammatory responses [40]. A study in the murine model revealed that the administration of anti-IL-2 neutralizing antibodies led to increased production of IL-1β by gut microbiota, subsequently influencing IL-2 production and orchestrating immune regulation in the intestine [41]. IL-17A is a major cytokine produced by Th17 cells, and its pathogenicity has been implicated in autoimmune diseases [42]. Both human and mouse studies have demonstrated that gut microbiota regulates the production of IL-17A through the influence of short-chain fatty acids (SCFAs) [43]. The metabolism of gut microbiota also plays a role in Th17-dependent autoimmunity in humans [44]. IL-22 has been implicated in various conditions involving inflammatory tissue pathology [45]. For instance, elevated IL-22 level was observed in colonic tissue of inflammatory bowel disease (IBD) patients, which indicated a pathologic role for IL-22 in IBD [45,46]. Moreover, signal transduction and activator of transcription 3 (STAT3) was activated by IL-22 signaling in human colon cancer cells, which indicated the role of IL-22 in malignant transformation [47]. Recent studies have reported associations between gut microbiota and IL-22, with SCFAs produced by gut microbiota contributing to IL-17 and IL-22 production [16,48–50]. [16,43,48]. Future studies should prioritize investigating the correlation between gut microbiota and various types of interleukins to uncover additional mechanisms underlying the interaction

between microbiota and inflammation.

The effects of interleukin therapy for AML patients were discussed in these years. Pro-inflammatory IL-1β and IL-6 tend to increase AML aggressiveness while anti-inflammatory mediators IL-10 appear to impede AML progression [51]. IL-6 is linked with altered microRNA expression correlated with leukemia in human and mice, and mediates the progression of myelodysplastic syndromes to AML [52,53]. Studies have discovered that blocking IL-6 can effectively reverse bone marrow failure and anemia, leading to improved overall survival in patients, making it a promising therapeutic approach. Additionally, IL-10 has been found to inhibit spontaneous AML blast proliferation in a majority of patients and decrease cytokine secretion from AML blast cells [54,55]. A recent study showed that IL-10 could promote the stemness of AML cells through the activation of the PI3K/AKT signal pathway [56]. As for the correlations between specific interleukins and AML, studies in the future should delve deeper into the interplay mechanisms between specific interleukins and AML.

Our study has revealed that IL-2 plays a mediating role in the associations between *Clostridiaceae 1*, *Clostridium sensu stricto 1*, and AML. Recent studies have extensively examined the effects of *Clostridium* and its various species on health and disease [57–62]. Notably, fecal Clostridium symbiosum, a species within the Clostridium genus, has shown promise as a biomarker for the early and noninvasive detection of colorectal cancer [60]. Previous studies also reported the interaction of *Clostridium* and interleukins [63–65]. Certain species of Clostridium have been found to regulate interleukin levels through the production of SCFAs via their metabolism [66,67]. Moreover, a human study demonstrated that prebiotics therapy can enhance the therapeutic outcome of IL-2 in cancer patients [68]. As a potential novel therapeutic strategy, future research should further validate the complex correlation between gut microbiota, interleukins, and AML in both animal models and human studies.

Our study aimed to explore the complicated interactive effects between gut microbiota, interleukins, and AML using a multinominal mediation MR analysis. MR analysis was based on a sample of 18,340 participants for gut microbiota, 3301 participants for interleukins, and 456,348 participants for AML, utilized F-statistics, potential confounders, and MR-PRESSO test to overcome the weak biases and horizontal pleiotropy. One limitation is that the AML data were mainly derived from populations of European descent whereas the gut microbiota data included populations of mixed ancestry. The findings of the current study should be extrapolated with caution. Future studies may perform metagenomic sequencing on gut microbiota, which could present stronger evidence among gut microbiota and AML [69].

Data availability statement

The summary-level data on gut microbiota (www.mibiogen.org), AML (GWAS GCST90042758), and interleukins (the INTERVAL study) used in this study are publicly and freely available in the GWAS database (https://gwas.mrcieu.ac.uk) and GWAS catalog (https://www.ebi.ac.uk/gwas/), with ethical approval and informed consent of participants for each cohort in the GWAS.

CRediT authorship contribution statement

Chenxi Luo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wei Zhang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jicheng Zhu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tianlai Qiu:** Writing – review & editing. **Qingbo Fang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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