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Dissecting the association between gut microbiota, body mass index and specific depressive symptoms: a mediation Mendelian randomisation study

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ABSTRACT

Background Observational studies highlight the association between gut microbiota (GM) composition and depression; however, evidence for the causal relationship between GM and specific depressive symptoms remains lacking.

Aims We aimed to evaluate the causal relationship between GM and specific depressive symptoms as well as the mediating role of body mass index (BMI).

Methods We performed a two-sample Mendelian randomisation (MR) analysis using genetic variants associated with GM and specific depressive symptoms from genome-wide association studies. The mediating role of BMI was subsequently explored using mediation analysis via two-step MR.

Results MR evidence suggested the *Bifidobacterium* genus (β=−0.03; 95% CI −0.05 to −0.02; p<0.001 and β=−0.03; 95% CI −0.05 to −0.02; p<0.001) and Actinobacteria phylum (β=−0.04; 95% CI −0.06 to −0.02; p<0.001 and β=−0.03; 95% CI −0.05 to −0.03; p=0.001) had protective effects on both anhedonia and depressed mood. The Actinobacteria phylum also had protective effects on appetite changes (β=−0.04; 95% CI −0.06 to −0.01; p=0.005), while the Family XI had an antiprotective effect (β=0.03; 95% CI 0.01 to 0.04; p<0.001). The Bifidobacteriaceae family (β=−0.01; 95% CI −0.02 to −0.01; p=0.001) and Actinobacteria phylum (β=−0.02; 95% CI −0.03 to −0.01; p=0.001) showed protective effects against suicidality. The two-step MR analysis revealed that BMI also acted as a mediating moderator between the Actinobacteria phylum and appetite changes (mediated proportion, 34.42%) and that BMI partially mediated the effect of the *Bifidobacterium* genus (14.14% and 8.05%) and Actinobacteria phylum (13.10% and 8.31%) on both anhedonia and depressed mood.

Conclusions These findings suggest a potential therapeutic effect of Actinobacteria and *Bifidobacterium* on both depression and obesity. Further studies are required to translate these findings into clinical practice.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous studies have implicated the gut microbiota (GM) composition in the pathogenesis of depression through the gut–brain axis. However, observational studies have yielded inconsistent findings, and no study has employed Mendelian randomisation to unveil a causal relationship between GM and specific depressive symptoms.

WHAT THIS STUDY ADDS

⇒ Our study constitutes a significant advancement in the field, with primary findings suggesting that (1) Actinobacteria and *Bifidobacterium* abundance exhibit a protective effect against anhedonia, depressed mood, appetite changes, and suicidality; and (2) body mass index acts as a mediator in the relationship between GM and anhedonia, depressed mood and appetite changes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings affirm the therapeutic efficacy of Actinobacteria and *Bifidobacterium* for depression, particularly in individuals exhibiting predominant symptoms of anhedonia, depressed mood and appetite changes. Furthermore, our results hint at the potential therapeutic advantage of probiotics for atypical depression or the co-occurrence of obesity and depression.

BACKGROUND

Major depression (MD) is a common, debilitating and multifaceted mental disorder that severely limits psychosocial functioning and contributes significantly to the worldwide disease burden.[1] Patients with MD exhibit typical symptoms, including emotional, physical and cognitive symptoms, yet the underlying mechanisms remain unknown. Traditionally, MD has been recognised as a dysfunction of brain processes; however, this brain-centric perspective overlooks the fact that the development and function of the nervous system are influenced by metabolic and immunological aspects of the body.[2] The

human intestine comprises a complex and diverse gut microbiota (GM) that could regulate brain function and behaviour by producing or modifying metabolic, neurochemical and immunological factors.[3] Accumulating evidence implicates the GM as playing a significant role in the pathogenesis of MD.

Contemporary studies, most of which were cross-sectional, have suggested that patients with MD have altered species of GM compared with healthy adults, including an elevated abundance of phylum Actinobacteria, family Bifidobacteriaceae and genus *Bifidobacterium* as well as a decrease in phylum Bacteroidetes, family Ruminococcaceae, genus *Faecalibacterium*.[3 4] For depressive symptoms, Mason and colleagues demonstrated a link between a decrease in anti-inflammatory GM and MD, particularly with regards to anhedonia.[5] Nevertheless, the association is unique to each study.[6] Confounding factors, including medication, dietary and lifestyle choices as well as reverse causal relationships, may be responsible for these discrepancies, which are common limitations in small observational studies.[6] Furthermore, inconsistent results could also be a consequence of the high heterogeneity of MD. Even though individuals may receive the same diagnosis of MD, their symptom profiles can vary significantly, and only a limited number of studies have found associations between specific depressive symptoms and GM. Importantly, understanding which group of GM influences one or more specific depressive symptoms may provide better guidance for clinical judgement regarding the appropriateness and type of probiotic administration therapy based on the patients' symptoms.

Randomised controlled trials (RCTs) of GM are complex, time-consuming and costly. Therefore, it is essential to approach causal inference in a rational manner to determine if such research is potentially warranted. Mendelian randomisation (MR) studies have been widely used to assess causal relationships by using genetic variants as instrument variables (IVs) of modifiable exposures. Genetic variants are randomly allocated during meiosis and fertilisation, and they are determined before the onset of the outcome. By employing MR analysis, bias due to confounding and reverse causation, as described above, can be minimised.[7] MR studies investigating the causal relationship between GM and MD have shown that an increase in Bacilli class is associated with an elevated risk of MD, while Actinobacteria, Bifidobacterium and the genus *Ruminococcus1* have a protective effect on MD.[8] Nonetheless, no MR analyses have examined the causal association between GM and specific depressive symptoms.

In summary, the highly heterogeneous nature of MD necessitates a focused investigation into GM and specific depressive symptoms. Existing cross-sectional studies have produced inconsistent results, and as of now, no MR study has delved into the causal relationship between GM and distinct depressive symptoms. To address this gap, we applied a two-sample MR analysis to evaluate the potential causal relationship between GM and specific depressive symptoms assessed by the Patient Health Questionnaire 9 (PHQ-9), which includes anhedonia, depressed mood, sleep disturbance, fatigue, appetite changes, low self-esteem, concentration problems, psychomotor changes and suicidality. Obesity is also a disease with a significant social burden and may contribute to the development of MD through increased body dissatisfaction.[9] Given previous evidence demonstrating the contribution of GM to obesity,[10] and the significant role of obesity in the development of MD, we used body mass index (BMI) as a mediator to investigate the mediating pathway from GM to MD via BMI. This study aimed to test two hypotheses. First, we hypothesised that the altered abundance of different GM groups leads to different depressive symptoms. Second, we hypothesised that BMI could act as a mediator between GM and specific depressive symptoms.

METHODS

Study design overview

We used summary-level data from large genome-wide association studies (GWAS) and implemented a two-sample mediation MR approach to dissect the association between GM, BMI and nine specific depressive symptoms in PHQ-9. Our analysis involved three main steps. First, we carried out two-sample MR analyses to investigate the causal effect of the association between GM and specific depressive symptoms. Second, we conducted two-sample MR analyses to evaluate the association between GM and BMI as well as the association between BMI and specific depressive symptoms. Finally, we employed a two-step mediation MR approach to ascertain the extent to which BMI mediates the causal association between GM and depressive symptoms. The flowchart of the current study is described in figure 1. The data sources for this study are detailed in online supplemental eTable 1.

Data sources

Gut microbiota

Genetic instruments of the GM were derived from the MiBioGen GWAS summary statistics.[11] This large-scale, multiethnic GWAS coordinated 16S ribosomal RNA gene sequencing profiles with genotyping data from 18 340 people across 24 cohorts, the vast majority (n=13 266) of whom are of European ancestry. After adjusting for age, principal genetic components, technical covariates, sex and 5 (mono-ethnic cohorts) or 10 (multi-ethnic cohorts) principal components, 122 110 genetic loci from 211 taxa were obtained. We excluded 15 bacterial traits without specific species name (unknown family or genus), leaving 196 bacterial traits for inclusion in the final MR analysis (119 genera, 32 families, 20 orders, 16 classes and 9 phyla).

Specific depressive symptoms

We used the UK Biobank GWAS summary data to assess depression symptoms, as measured by the PHQ-9 in an online questionnaire.[12] The PHQ-9 is designed to identify

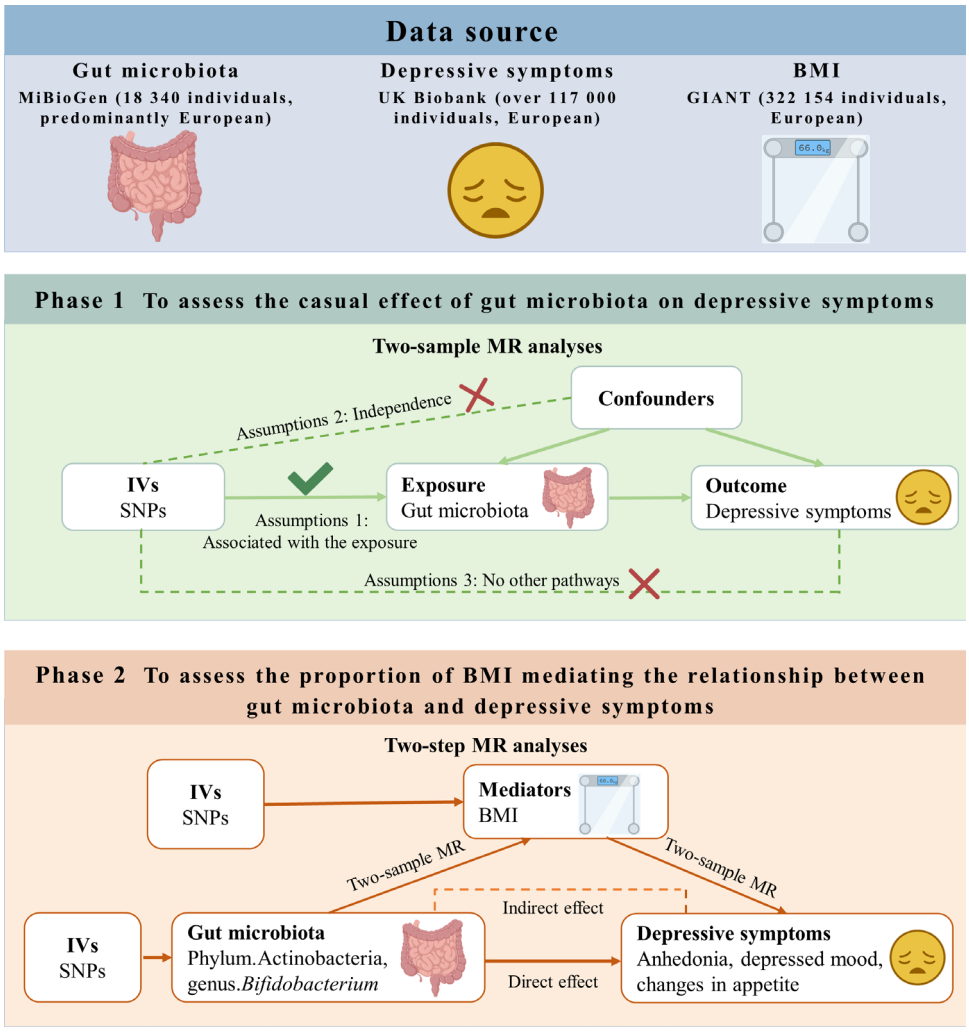

Figure 1 Overview of the study design. In this study, two analytical phases were involved. In phase 1, two-sample MR analyses were conducted to investigate the causal effect of the association between gut microbiota and specific depressive symptoms. In phase 2, a two-step mediation MR approach was used to determine the extent to which BMI mediates the causal association between gut microbiota and depressive symptoms. BMI, body mass index; IVs, instrument variables; MR, Mendelian randomisation; SNPs, single nucleotide polymorphisms.

depressive symptoms as defined by the Diagnostic and Statistical Manual of Mental Disorders-IV.[13] The questionnaire inquires if any of the nine symptoms have bothered the respondents in the previous 2weeks. Responses are categorised into four levels based on the frequency of symptoms. The Neale lab performed a GWAS for depressive symptoms using PHQ-9 data that were corrected for age, age squared, sex, age*sex, age squared*sex, and the first 20 main components of genotyping data. Further information about the UK Biobank data on specific depressive symptoms is presented in online supplemental eTable 2.

Body mass index

We extracted summary statistics of GWASs associated with BMI from the GIANT consortium, which included 322 154 European individuals, with covariates such as gender, age, age squared and principal components.[14] The GWAS data do not include UK Biobank participants to ensure sample independence.

Selection of instrumental variables

IVs were selected based on the following criteria: (1) the GWAS-correlated p value was set to 1×10^{-5} to obtain a more comprehensive result for GM,[15] while the threshold for BMI was set to 5×10^{-8}; (2) the clumping process (GM: $r^2<0.1$, clumping distance=500 kb; BMI: $r^2<0.001$, clumping distance=10 000 kb) was performed to assess the linkage disequilibrium between single-nucleotide polymorphisms (SNPs); (3) to prevent weak IV issues that could result in biased effect estimates, SNPs with an F statistic <10 were excluded. The F statistics were calculated using the formula $F=\frac{R^2\times(n-2)}{1-R^2}$, where $R^2=\frac{2\times\beta^2\times EAF\times(1-EAF)}{2\times\beta^2\times EAF\times(1-EAF)+2\times SE^2\times N\times EAF\times(1-EAF)}$; (4) when SNPs related to exposure were absent in the GWAS outcome data, we replaced them with proxy SNPs ($r^2>0.8$) using the linkage disequilibrium proxy search online (https://ldlink.nci.nih.gov/) and (5)

harmonisation to exclude ambiguous and palindromic SNPs. More details of the selected IVs are presented in online supplemental eTable 3, eTable 4.

Statistical analyses

Two-sample MR

Based on the IVs selected above, we performed a two-sample MR analysis in R, V.4.2.2, using the TwoSampleMR and MRPRESSO packages. The inverse variance weighted (IVW) method was employed as the primary MR analysis, which combined the Wald ratio estimates of each SNP on the outcome and obtained a pooled causal estimate. The IVW method offers the most accurate estimates, assuming the validity of all SNPs, although it is susceptible to potential pleiotropic effects.[16] To enhance the robustness of our findings, two supplementary approaches were employed alongside IVW: the weighted median and MR-Egger

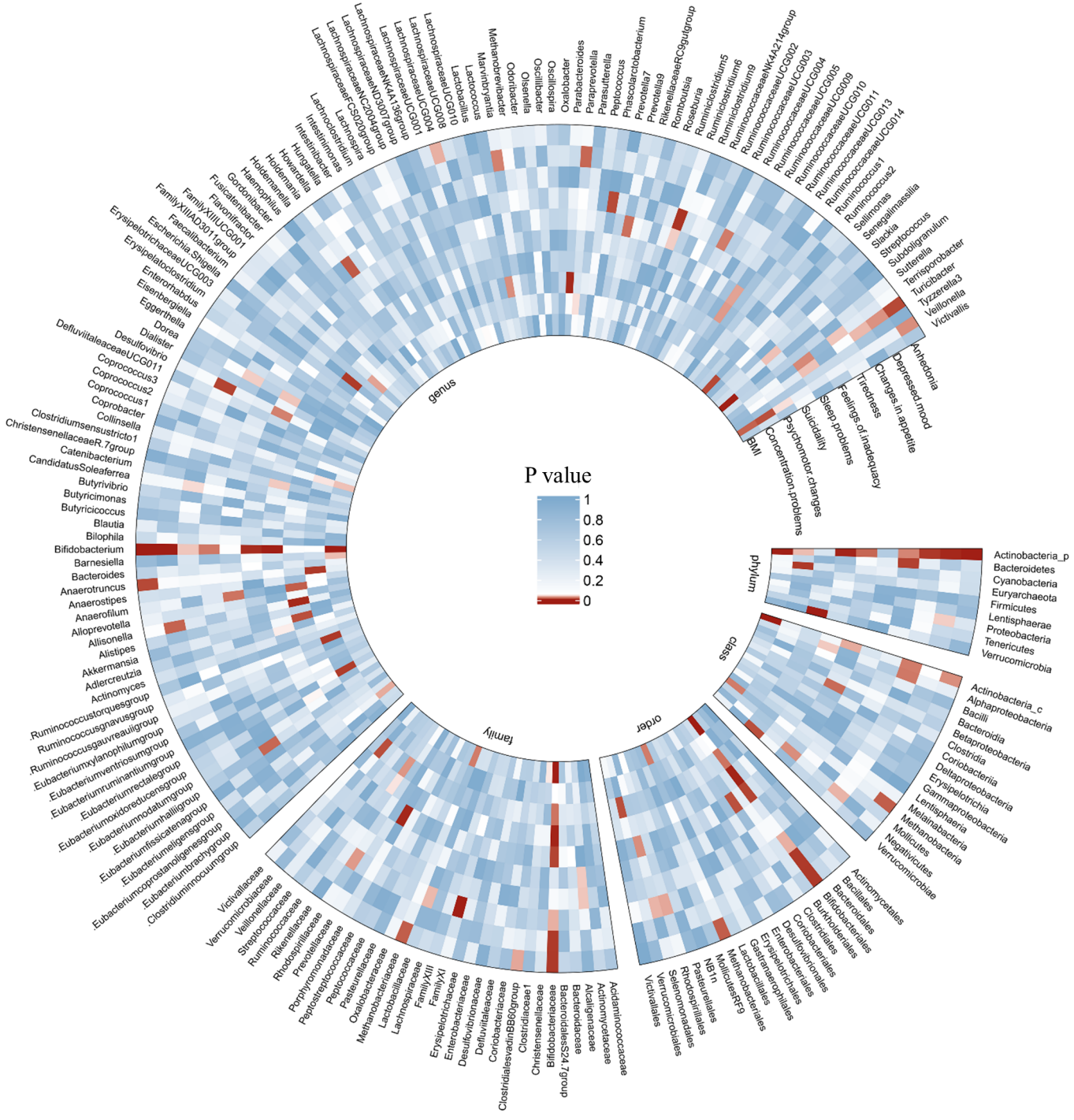

Figure 2 Preliminary associations between gut microbiota and specific depressive symptoms and body mass index derived using the inverse variance weighted method. Within the heatmap, bacterial traits are sequentially classified into five categories (phylum, class, order, family and genus) based on their feature level. Estimates with p<0.05 were shown in red, and those with p>0.05 were shown in blue.

methods. The weighted median method delivers consistent estimates when more than 50% of the weight comes from valid instrument variants. In contrast, MR-Egger regression provides correct estimates even when all instruments are invalid, but it necessitates variants to satisfy the instrument strength independent of direct effects assumption. If the IVW method yields a significant result without pleiotropy or heterogeneity, even if the other two methods do not, it can be considered a positive result, provided that the beta direction is consistent in all MR methods.[17]

The results were then subjected to a series of sensitivity analyses. To evaluate the stability of the results, the potential effect of each SNP on the outcomes was detected using the leave-one-out approach; second, the Cochrane's Q test was computed to detect heterogeneity, with IVs having a p value <0.05 considered heterogeneous. Finally, the intercept of MR-Egger and MR pleiotropy residual sum and outlier method (MR-PRESSO) was used to monitor the potential horizontal pleiotropy effect. If pleiotropy is detected, MR analyses need to be repeated after removing the outliers. We did not perform a reverse MR analysis of GM and specific depressive symptoms due to a lack of validated IVs for depressive symptoms summary data.

In the MR analysis between GM and specific depressive symptoms, the Bonferroni correction was used to adjust for multiple comparisons at each feature level (phylum, class, order, family and genus), with a cut-off of p<0.05/n (n represents the number of bacterial traits at the corresponding level).

Mediation analysis

We applied a two-step MR analysis to assess the mediating effect of BMI. In the first step, IVs for GM were used to estimate the causal effect on BMI, and in the second step, IVs for BMI were used to estimate the causal effect on depressive symptoms. The results of the first step were corrected using the Bonferroni correction described above. Subsequently, we conducted mediation analyses using the product of coefficient method,[18] with BMI as the independent variable (X), GM as the mediating variable (M) and MD as the dependent variable (Y). Path a (effect of X to M) and path b (effect of M to Y) were calculated by regression analysis, and then the mediating effect of BMI on the relationship between GM and MD was assessed by the product a×b. 95% CIs for indirect effects were computed by using the delta method.[18]

RESULTS

Associations of GM with specific depressive symptoms

The results of MR analyses investigating the association between 196 types of GM and nine specific depressive symptoms are shown in figure 2. The IVW results revealed several significant associations between GM and depressive symptoms (table 1). As for the phylum classifications, Actinobacteria demonstrated a protective effect against anhedonia (β=–0.04; 95% CI –0.06 to –0.02; p<0.001), depressed mood (β=–0.03; 95% CI –0.05 to –0.03; p=0.001), appetite changes (β=–0.04; 95% CI –0.06 to –0.01; p=0.005) and suicidality (β=–0.02; 95% CI –0.03 to –0.01; p=0.001). Furthermore, results also indicated that Cyanobacteria was a risk factor for concentration problems (β=0.03; 95% CI 0.01 to 0.05; p=0.005), while Verrucomicrobia demonstrated a protective effect against psychomotor changes (β=–0.03; 95% CI –0.04 to –0.01; p<0.001). In terms of family classifications, the host genetic-driven increase in Family XI was related to a higher risk of appetite changes (β=0.03; 95% CI 0.01 to 0.04; p<0.001), whereas Bifidobacteriaceae was related to a lower risk of suicidality (β=–0.01; 95% CI –0.02 to –0.01; p=0.001). Finally, concerning genus classifications, *Bifidobacterium* showed a protective effect against anhedonia (β=–0.03; 95% CI –0.05 to –0.02; p<0.001) and depressed mood (β=–0.03; 95% CI –0.05 to –0.02; p<0.001). Scatter plots and forest plots of all MR analyses results with

Table 1 Mendelian randomisation results of causal effects between gut microbiome and depressive symptoms

Gut microbiota (exposure)	Depressive symptoms (outcome)	Number of SNPs	β (95% CI)	P
Genus.*Bifidobacterium*	Anhedonia	19	–0.03 (–0.05 to –0.02)	<0.001
Phylum.Actinobacteria	Anhedonia	19	–0.04 (–0.06 to –0.02)	<0.001
Genus.*Bifidobacterium*	Depressed mood	19	–0.03 (–0.05 to –0.02)	<0.001
Phylum.Actinobacteria	Depressed mood	19	–0.03 (–0.05 to –0.03)	0.001
Family.Family XI	Changes in appetite	8	0.03 (0.01 to 0.04)	<0.001
Phylum.Actinobacteria	Changes in appetite	19	–0.04 (–0.06 to –0.01)	0.005
Family.Bifidobacteriaceae	Suicidality	20	–0.01 (–0.02 to –0.01)	0.001
Phylum.Actinobacteria	Suicidality	19	–0.02 (–0.03 to –0.01)	0.001
Phylum.Cyanobacteria	Concentration problems	8	0.03 (0.01 to 0.05)	0.005
Phylum.Verrucomicrobia	Psychomotor changes	12	–0.03 (–0.04 to –0.01)	<0.001

β, the effect size of the exposure on depressive symptoms, the effect size was derived by IVW.
IVW, inverse variance weighted; SNPs, single nucleotide polymorphisms.

significant associations between GM and depressive symptoms are presented in online supplemental eFigure 1. Notably, we found no significant associations in either the class or order classification.

Sensitivity analysis

The sensitivity analysis was detailed in online supplemental eFigure 3. As complementary approaches, the results of the MR-Egger, weighted mode, simple mode and weighted median methods supported the findings from the IVW method, with consistent beta direction (online supplemental eTable 5). The Cochrane Q statistics revealed no significant heterogeneity across instrument effect (online supplemental eTable 6). Furthermore, utilising the MR-Egger regression intercept method and the MRPRESSO analysis, we observed no evidence of horizontal pleiotropy for GM in depressive symptoms with p>0.05 (online supplemental eTable 6). The results of IVW were reliable in the absence of heterogeneity and pleiotropy. Finally, the leave-one-out results confirmed the data's robustness (online supplemental eFigure 2).

Mediation analysis

Given the evidence linking obesity to both depressive symptoms and GM, we performed a two-step MR analysis to investigate the mediating pathway from GM to depressive symptoms via BMI. In the first step, we used genetic instruments for GM to assess the causal effect on the mediators, BMI. We found that the Phylum Actinobacteria (β=–0.08; 95% CI –0.13 to –0.05; p<0.001), Class Actinobacteria (β= –0.09; 95% CI –0.13 to –0.05; p<0.001), Order Bifidobacteriales (β=–0.09; 95% CI –0.13 to –0.06; p<0.001), Family Bifidobacteriaceae (β=–0.09; 95% CI –0.13 to –0.06; p<0.001) and Genus *Bifidobacterium* (β=–0.09; 95% CI –0.13 to –0.05; p<0.001) were all negatively associated with BMI, suggesting a protective effect against obesity (figure 2, figure 3B, online supplemental eTable 7 and eFigure 4). In the second step, we assessed the causal association between BMI and nine specific depressive symptoms. The genetic instruments for BMI indicated that higher BMI was associated with anhedonia (β=0.05; 95% CI 0.03 to 0.08; p<0.001), depressed mood (β=0.03; 95% CI 0.01 to 0.06; p=0.017), changes in appetite (β=0.14; 95% CI 0.11 to 0.17; p<0.001) and feelings of tiredness (β=0.06; 95% CI 0.01 to 0.10; p=0.013) and inadequacy (β=0.03; 95% CI 0.01 to 0.16; p=0.008) (figure 3C, figure 3D, online supplemental eTable 8 and eFigure 5).

Finally, in conjunction with the above findings, we identified five mediating effects (figure 3A and table 2). Namely, the Phylum Actinobacteria and Genus *Bifidobacterium* could reduce the risk of anhedonia by reducing BMI (β=–0.01; 95% CI –0.00 to –0.01 and β=–0.01; 95% CI –0.00 to –0.01), with mediating percentages of 13.10% and 14.14%, respectively. The Phylum Actinobacteria and Genus *Bifidobacterium* could also reduce the risk of depressed mood by reducing BMI (β=–0.00; 95% CI 0.00 to –0.01 and β=–0.00; 95% CI 0.00 to –0.01), with mediating percentages of 8.31% and 8.05%, respectively. Furthermore, the Phylum Actinobacteria could reduce the risk of appetite changes by reducing BMI (β=–0.01; 95% CI –0.01 to –0.02), with mediating percentages of 34.42%.

DISCUSSION

Main findings

In this two-step mediation MR study, we first found suggestive evidence of causal relationships between the *Bifidobacterium* genus and Actinobacteria phylum with both anhedonia and depressed mood, the Family XI and Actinobacteria phylum with appetite changes, the Bifidobacteriaceae family and Actinobacteria phylum with suicidality, the Cyanobacteria phylum with concentration problems and the Verrucomicrobia phylum with psychomotor changes. In the other direction, our results indicated that Actinobacteria phylum, encompassing the Actinobacteria class, Bifidobacteriaies order, Bifidobacteriaceae family and *Bifidobacterium* genus could potentially alter BMI, while alterations in BMI could influence the risk of anhedonia, depressed mood, appetite changes, feelings of tiredness and inadequacy. Subsequently, we conducted a mediation analysis. The results illustrated that the effect of *Bifidobacterium* genus and Actinobacteria phylum on both anhedonia and depressed mood is partially mediated by BMI. BMI was also identified as a mediating moderator between Actinobacteria phylum and appetite changes. To our knowledge, this is the first MR study to dissect the casual relationship between GM and specific depressive symptoms as well as to determine the mediating role of BMI in this.

The phylum Actinobacteria is ubiquitous in nature and consists of three major anaerobe families (Bifidobacteriaceae, Propionibacteria, and Corynebacteria). *Bifidobacterium*, classified in the Bifidobacteriaceae family, is known for its beneficial effects in various pathological conditions. Our findings indicate that Actinobacteria or *Bifidobacterium* have a protective effect against anhedonia, depressed mood, appetite changes, and suicidality. Counterintuitively, existing human observational studies have produced inconsistent findings, with the majority concluding that the abundance of Actinobacteria, including the family Bifidobacteraceae and the genus *Bifidobacterium*, is positively associated with MD or negative mood.[4 19] Only two studies support our findings, suggesting a protective effect of such microbiota against depression or anhedonia.[5 20] This discrepancy may be attributed to confounding factors in observational studies (eg, dietary composition, medication use and lifestyle) and reverse causality. In contrast, MR studies can minimise this bias. Nevertheless, several studies have demonstrated that *Bifidobacterium*, when administered as probiotics, may exhibit antidepressant effects.[21 22] Notably, one study found that the antidepressant effect was associated with reduced limbic reactivity to negative emotional stimuli,[22] suggesting that *Bifidobacterium* may shape the brain through the microbiota–gut–brain axis. Furthermore, our findings align with an animal study showing that *Bifidobacterium* can increase brain-derived neurotrophic factor levels and prevent or improve depressive behaviours in mice.[23] In summary, our findings contradict most existing clinical observational

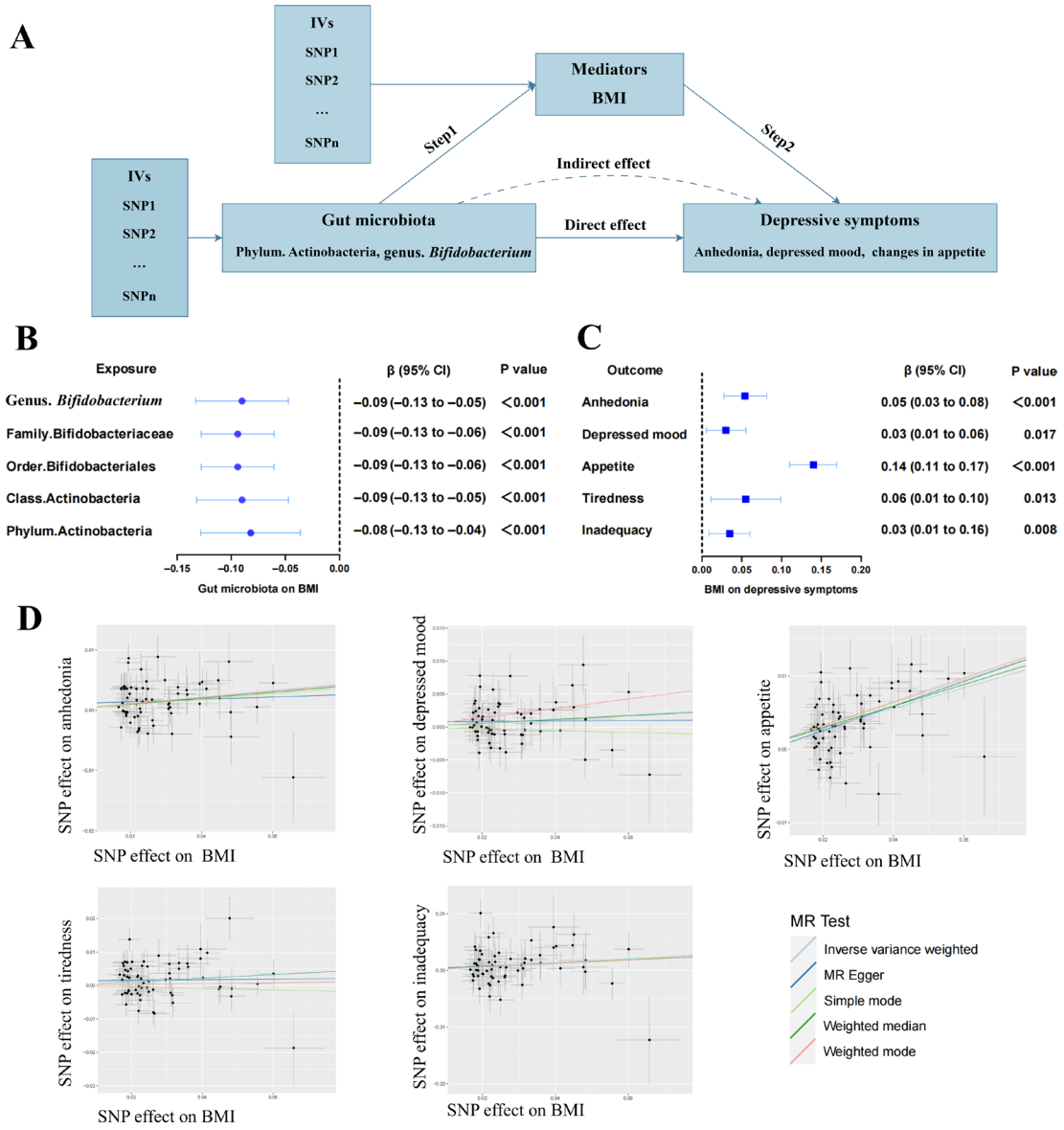

Figure 3 Mediation analysis of the effect of gut microbiota on depressive symptoms via BMI. (A) outlines the two-step MR analysis framework. Step 1 estimated the causal effect of the gut microbiota on the BMI, and step 2 assessed the causal effect of the BMI on depressive symptoms. 'Direct effect' refers to the effect of gut microbiota on depressive symptoms after adjusting for BMI. 'Indirect effect' indicates the effect of gut microbiota on depressive symptoms through BMI. (B) displays MR estimates, derived from the IVW method, which illustrate the effect of gut microbiota on BMI. (C) presents MR estimates, derived using the IVW method, which illustrate the effect of BMI on depressive symptoms. The error bars denote 95% CIs and p<0.05 is considered indicative of a significant association. (D) presents the scatter plot of the effect of BMI on depressive symptoms. BMI, body mass index; IVs, instrument variables; IVW, inverse variance weighted; MR, Mendelian randomisation; SNP, single nucleotide polymorphism.

studies but align with the results of human and animal probiotic treatments. We propose that Actinobacteria, specifically *Bifidobacterium*, may reduce the risk of depressive symptoms such as anhedonia, depressed mood, appetite changes and suicidality. In the clinical setting, *Bifidobacterium* has been investigated as a probiotic for managing MD.[21 22] Our study not only validates its therapeutic impact on MD but also suggests potential benefits for specific symptoms such as anhedonia, depressed mood, appetite changes and suicidality. Notably, these effects appear particularly pronounced in patients exhibiting significant depressive symptoms. Looking ahead, we propose a potential paradigm shift in future clinical practice, where depression could be stratified into subgroups based on specific symptoms. This stratification might reveal whether probiotics, specifically *Bifidobacterium*, exhibit enhanced effectiveness for subgroups with distinct symptoms. Consequently, our findings carry crucial implications for optimising the clinical efficacy of *Bifidobacterium* therapy.

We found that phylum Verrucomicrobia had protective effects on psychomotor changes, while Cyanobacteria were identified as risk factors for concentration problems.

Table 2 The mediation effect of gut microbiome on depressive symptoms via body mass index

Gut microbiota (exposure)	Depressive symptoms (outcome)	Total effect β (95% CI)	Direct effect A β (95% CI)	Direct effect B β (95% CI)	Mediation effect β (95% CI)	Mediated proportion (%)
Genus. *Bifidobacterium*	Anhedonia	−0.03 (−0.05 to −0.02)	−0.09 (−0.13 to −0.05)	0.05 (0.03 to 0.08)	−0.01 (−0.00 to −0.01)	14.14
Phylum. Actinobacteria	Anhedonia	−0.04 (−0.06 to −0.02)	−0.09 (−0.13 to −0.04)	0.05 (0.03 to 0.08)	−0.01 (−0.00 to −0.01)	13.10
Genus. *Bifidobacterium*	Depressed mood	−0.03 (−0.05 to −0.02)	−0.09 (−0.13 to −0.05)	0.03 (0.01 to 0.06)	−0.00 (−0.00 to −0.01)	8.05
Phylum. Actinobacteria	Depressed mood	−0.03 (−0.05 to −0.03)	−0.09 (−0.13 to −0.04)	0.03 (0.01 to 0.06)	−0.00 (−0.00 to −0.01)	8.31
Phylum. Actinobacteria	Changes in Appetite	−0.04 (−0.06 to −0.01)	−0.09 (−0.13 to −0.04)	0.14 (0.11 to 0.17)	−0.01 (−0.01 to −0.02)	34.42

'Total effect' refers the effect of gut microbiota on major depression, 'direct effect A' indicates the effect of gut microbiota on body mass index, 'direct effect B' indicates the effect of body mass index on major depression and 'mediation effect' indicates the effect of gut microbiota on major depression through body mass index. Total effect, direct effect A and direct effect B were derived by inverse variance weighted; mediation effect was derived by using the delta method.

Although evidence for the association between Verrucomicrobia and MD in humans is still lacking, Sun and colleagues identified that Verrucomicrobia was reduced in a chlorpyrifos-induced depression mouse model.[24] Furthermore, recent research has shown that Akkermansia muciniphila, a Verrucomicrobia next-generation probiotic, may reduce depressive symptoms in mice by modulating GM and metabolites.[25] However, previous research contradicts our findings, indicating that Cyanobacteria is a beneficial bacteria capable of exerting antidepressant effects.[26] This difference may be because Cyanobacteria primarily impact concentration problems, which alone may not be sufficient to affect the onset of MD. More RCTs of Cyanobacteria and specific depressive symptoms are, therefore, needed to clarify their functions. The clinical evidence regarding the antidepressant effects of Verrucomicrobia, in comparison to *Bifidobacterium*, remains insufficient. Our causal inference findings suggest a potential protective effect against psychomotor changes. Consequently, we propose exploring the translation of these insights into animal experiments to clinical trials with small sample sizes. This approach could be particularly promising for patients with depression, especially those with prominent psychomotor changes.

The subsequent two-step mediation analysis revealed that an increased abundance of Actinobacteria and *Bifidobacterium* could reduce the risk of anhedonia and depression by decreasing BMI. Furthermore, increased Actinobacteria abundance may also reduce the risk of appetite changes by reducing BMI. In the first MR step, we found that the abundance of Actinobacteria and *Bifidobacterium* was negatively correlated with BMI. Consistent with our results, an observational study by Ignacio and colleagues reported a negative correlation between BMI and *Bifidobacterium* copy number.[27] The second MR step provided evidence that a higher BMI increases the risk of anhedonia, depressed mood, appetite changes, feelings of inadequacy and tiredness. MR studies have consistently demonstrated that a higher BMI is associated with an increased risk of depression.[28] Moreover, emerging evidence suggests that BMI correlates more strongly with MD symptoms labelled as 'atypical', including fatigue, excessive sleepiness, weight gain and mood reactivity.[29] Although there is sufficient clinical evidence supporting a correlation between obesity and depressive symptoms, the underlying biological mechanisms remain unclear. Our findings suggest that decreased abundance of Actinobacteria and *Bifidobacterium* elevates the risk of obesity and depressive symptoms, and that BMI mediates the causal relationship between GM and anhedonia, depressed mood and appetite changes, although the indirect effect was less pronounced than the total effect. Both MD and obesity are major contributors to the global burden of disease. Our results confirm that Actinobacteria, especially *Bifidobacterium*, may be protective against both diseases. In future clinical research, the efficacy of *Bifidobacterium* for both disorders can be further observed in patients with MD characterised by 'atypical' symptoms or MD comorbid obesity, which is suggestive for optimising the efficacy of MD treatment.

Limitations

There are several limitations that need to be emphasised. First, the data on depressive symptoms used in this study do not differentiate between the general population and patients with MD. In fact, depressive symptoms in these two populations may exist on a continuum or have different underlaying mechanisms and implications. This may have affected the scalability of our findings in patients with MD. Therefore, even if our results demonstrate a potential therapeutic role of probiotics for depression, future MR analyses and RCTs specifically targeting patients with MD are needed to validate the robustness of our findings. Additionally, it is crucial to highlight that the GWAS data primarily involved participants of European descent.

Consequently, potential interference from population stratification should be considered. The findings may not fully extend to individuals of non-European ancestry, posing a challenge to the scalability of our results. Despite substantial evidence linking higher BMI to elevated depression rates in individuals of European descent, a recent MR study indicates an inverse correlation between BMI and depression in those of East Asian ancestry.[30] However, the limited data available have left the association between GM and MD unexplored in diverse ancestral populations. Hence, it is imperative to independently validate our results in cohorts representing various ethnicities.

Implications

This two-sample MR study provides a comprehensive investigation into the association between GM, BMI and specific depressive symptoms using large GWAS data. Our primary findings demonstrate a protective association between the abundance of Actinobacteria and *Bifidobacterium* and a lower risk of anhedonia, depressed mood, appetite changes and suicidality. Furthermore, BMI mediated the effect between GM and anhedonia, depressed mood and appetite changes. The implications of our findings extend to the potential therapeutic role of probiotics in addressing depression, offering a simultaneous impact on both depression and obesity, two conditions imposing a substantial societal burden. However, it is crucial to note that further validation through replication in diverse populations and large-scale RCTs is imperative before considering clinical implementation.

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REFERENCES

1 Malhi GS, Mann JJ. Depression. *The Lancet* 2018;392:2299–312.
2 Morais LH, Schreiber HL 4th, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. *Nat Rev Microbiol* 2021;19:241–55.
3 Modesto Lowe V, Chaplin M, Sgambato D. Major depressive disorder and the gut microbiome: what is the link? *Gen Psychiatr* 2023;36:e100973.
4 Zheng P, Zeng B, Zhou C, *et al*. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry* 2016;21:786–96.
5 Mason BL, Li Q, Minhajuddin A, *et al*. Reduced anti-inflammatory gut microbiota are associated with depression and anhedonia. *J Affect Disord* 2020;266:394–401.
6 Knuesel T, Mohajeri MH. The role of the gut microbiota in the development and progression of major depressive and bipolar disorder. *Nutrients* 2021;14:37.
7 Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ* 2018;362:k601.
8 Chen M, Xie C-R, Shi Y-Z, *et al*. Gut microbiota and major depressive disorder: a bidirectional Mendelian randomization. *J Affect Disord* 2022;316:187–93.
9 Blundell E, De Stavola BL, Kellock MD, *et al*. Longitudinal pathways between childhood BMI, body dissatisfaction, and adolescent depression: an observational study using the UK Millenium Cohort Study. *Lancet Psychiatry* 2024;11:47–55.
10 Gomes AC, Hoffmann C, Mota JF. The human gut microbiota: metabolism and perspective in obesity. *Gut Microbes* 2018;9:308–25.
11 Kurilshikov A, Medina-Gomez C, Bacigalupe R, *et al*. Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat Genet* 2021;53:156–65.
12 Davis KAS, Coleman JRI, Adams M, *et al*. Mental health in UK Biobank - development, implementation and results from an online questionnaire completed by 157 366 participants: a reanalysis. *BJPsych Open* 2020;6:e18.
13 Löwe B, Unützer J, Callahan CM, *et al*. Monitoring depression treatment outcomes with the patient health questionnaire-9. *Med Care* 2004;42:1194–201.
14 Locke AE, Kahali B, Berndt SI, *et al*. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015;518:197–206.

15 Sanna S, van Zuydam NR, Mahajan A, *et al*. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat Genet* 2019;51:600–5.
16 Yuan S, Chen J, Ruan X, *et al*. Smoking, alcohol consumption, and 24 gastrointestinal diseases: Mendelian randomization analysis. *Elife* 2023;12:e84051.
17 Cheng F, Luk AO, Shi M, *et al*. Shortened leukocyte telomere length is associated with glycemic progression in type 2 diabetes: a prospective and Mendelian randomization analysis. *Diabetes Care* 2022;45:701–9.
18 VanderWeele TJ. Mediation analysis: a practitioner's guide. *Annu Rev Public Health* 2016;37:17–32.
19 Kurokawa S, Kishimoto T, Mizuno S, *et al*. The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: an open-label observational study. *J Affect Disord* 2018;235:506–12.
20 Aizawa E, Tsuji H, Asahara T, *et al*. Possible association of bifidobacterium and lactobacillus in the gut microbiota of patients with major depressive disorder. *J Affect Disord* 2016;202:254–7.
21 Tian P, Chen Y, Zhu H, *et al*. Bifidobacterium breve CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: a randomized clinical trial. *Brain Behav Immun* 2022;100:233–41.
22 Pinto-Sanchez MI, Hall GB, Ghajar K, *et al*. Probiotic bifidobacterium longum Ncc3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. *Gastroenterology* 2017;153:448–59.
23 Kosuge A, Kunisawa K, Arai S, *et al*. Heat-sterilized bifidobacterium breve prevents depression-like behavior and Interleukin-1B expression in mice exposed to chronic social defeat stress. *Brain Behav Immun* 2021;96:200–11.
24 Sun Y, Pei J, Chen X, *et al*. The role of the gut Microbiota in depressive-like behavior induced by Chlorpyrifos in mice. *Ecotoxicol Environ Saf* 2023;250:114470.
25 Guo D, Park C, Li Y, *et al*. Akkermansia muciniphila ameliorates depressive disorders in a murine alcohol-LPS (mALPS) model. *Food Funct* 2022;13:12766–76.
26 Chi L, Khan I, Lin Z, *et al*. Fructo-oligosaccharides from morinda officinalis remodeled gut microbiota and alleviated depression features in a stress rat model. *Phytomedicine* 2020;67:153157.
27 Ignacio A, Fernandes MR, Rodrigues VAA, *et al*. Correlation between body mass index and faecal microbiota from children. *Clin Microbiol Infect* 2016;22:258.
28 Kappelmann N, Arloth J, Georgakis MK, *et al*. Dissecting the association between inflammation, metabolic dysregulation, and specific depressive symptoms: a genetic correlation and 2-sample mendelian randomization study. *JAMA Psychiatry* 2021;78:161–70.
29 Lasserre AM, Strippoli M-PF, Glaus J, *et al*. Prospective associations of depression subtypes with cardio-metabolic risk factors in the general population. *Mol Psychiatry* 2017;22:1026–34.
30 O'Loughlin J, Casanova F, Fairhurst-Hunter Z, *et al*. Mendelian randomisation study of body composition and depression in people of East Asian ancestry highlights potential setting-specific causality. *BMC Med* 2023;21:37.

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