Multi-cohort analysis of depression-associated gut microbiota sheds insight on bacterial biomarkers across populations

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Abstract

Gut microbes are associated with the development of depression based on extensive evidence. However, previous studies have led to conflicting reports on this association, posing challenges to the application of the gut microbiota in the diagnostics and treatment of depression. To minimize heterogenicity in data analysis, the present meta-analysis adopted a standardized bioinformatic and statistical pipeline to analyze 16s rRNA sequences of 1827 samples from 8 different cohorts. Although changes in the overall microbial community were identified by our meta-analysis, depressive-correlated changes in alpha-diversity were absent. Enrichment of Bacteroidetes, *Parabacteroides*, *Barnesiella*, *Bacteroides*, *Alistipes inops*, *Bacteroides massiliensis*, along with depletion in Firmicutes, *Dialister* and *Bacteroides plebeius* were observed in depressive-associated microbiota. By contrast, elevated L-glutamine degradation and reduced L-glutamate production and L-isoleucine biosynthesis were identified in depressive-associated microbiome. After systemically reviewing the data of these collected cohorts, we have established a microbial classifier to identify depressive symptoms with AUC > 78%. Moreover, a low-risk microbial cluster for depressive symptoms was identified, which presented by a lower abundance of *Escherichia-Shigella*, and a higher abundance of *Faecalibacterium*, Oscillospiraceae UCG 002, *Ruminococcus* and Christensenellaceae R.7 group.

Introduction

Depression is a common mental disorder that can persist for life. The aetiology of depression is related to multiple factors; personality, genetics, biochemical imbalances and environmental factors, such as stress, can collectively drive the development of depression. Over 200 million of the world population are diagnosed with depression[1], with a lifetime prevalence of 20%-25% in women and 7%-12% in men[2]. The manifestation of depression often includes emotional, behavioural, and cognitive dysregulations, stunted emotional responses and, consequently, poor function at work, school and home. Depression can also be complicated by its tight connection to neurological disorders such as dementia and Alzheimer's disease, further emphasizing its great impact on public health. However, depression is widely undiagnosed and untreated due to social stigma, limited accessibility to resources and a lack of effective therapies[2, 3]. Although there is an increasing awareness of the limited efficacy and adverse effects of the current diagnoses[4] and therapies[5], further research is required to improve patients' care.

Gut microbiota is a collection of microbes that colonize the gastrointestinal (GI) tract: it is functionally associated with the host in a symbiotic relationship that regulates the host physiology via metabolic and immunological activities. The gut microbiota has been implicated in the development of obesity[6], type 2 diabetes, non-alcoholic liver disease and cardio-metabolic diseases[7]. The interaction between the hypothalamic-pituitary-adrenal (HPA) system and the gut microbiota, termed the gut-brain axis, has been established in a mice model study[8], revealing a putative regulation of the neuropsychological behaviour by gut microbiota. Thus, scores of studies on the gut-brain axis have documented its involvement in immune-regulation, neuroendocrine, and vagus nerve pathways. However, variations in study design, sample populations and, most importantly, data analysis techniques, make it hard to summarise and

interpret findings from multiple sources. A recent systematic review reported a reduction in the abundance of *Bacteroidetes, Prevotellaceae, Faecalibacterium, Coprococcus, Sutterella* and an increase in the abundance of *Actinobacteria and Eggerthella* among participants with major depressive disorder (MDD)/depressive disorders compared to the healthy controls[9]. However, such changes in the microbial composition were not reported as significant by a large number of other studies[9, 10]. Establishing a standard approach to enable better comparisons between studies is needed[11] to reduce heterogeneity and gain insight into the association between gut microbiota and depression. Here we conducted a systematic multi-cohort analysis of gut microbiota using a standardized bioinformatic pipeline, thereby identifying consistent microbiota markers across cohorts, providing novel insights on their potential value in diagnosing depression.

Materials And Methods

Search strategy

The protocol for this systematic review and meta-analysis was registered at the International Prospective Register of Systematic Reviews (PROSPERO), under CRD42020206369. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were used as guidelines for article searches and filtering.

Article searches were performed by searching the PubMed, Scopus, and Web of Science databases for relevant English literature on Aug 5th, 2019. The search strategy in PubMed was:

(("Depression"[MeSH]) OR "Depressive Disorder"[MeSH]) AND "Gastrointestinal Microbiome"[MeSH]; in Scopus was: TITLE-ABS-KEY (depression AND "Gut microbiota") AND DOCTYPE (ar OR re) AND (LIMIT-TO (DOCTYPE, "ar")); in Web of Science was Theme: ("gut microbiota" AND "depression"), Filter: Type: (ARTICLE OR LETTER), Time: All. INDEX: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI.

Peer-reviewed papers were considered, regardless of publication year.

Moreover, the reference lists of the identified original articles and reviews were also reviewed manually for additional studies that may have been missed.

Eligibility criteria

Articles were included if they met the following criteria: 1) The included studies must be original studies; 2) participants in intervention/exposure group must have symptoms of depression or have higher depression scores compared with the control group; 3) the study must have analyzed the gut microbiota community via High-throughput sequencing, including 16S and or shotgun sequencing; 4) The articles must be published as full-text articles in peer-reviewed journals in English.

Articles were excluded if the articles were published as reviews, case reports, abstracts, or protocols. Articles without human samples were also excluded from the final systematic review and meta-analysis.

Study selection

The literature search results from different databases were combined in Endnote, where duplicate entries were deleted. Two stages of screening were performed for article filtering: Firstly, their titles and/or abstracts were screened independently by two review authors (S.L and Z.S) to identify studies that meet the eligibility criteria. Next, the potentially eligible studies were subjected to full-text screening independently by S.L and Z.S. Any disagreement between them over the eligibility of particular studies was resolved through discussion with a third reviewer (K.P).

Data extraction

The characteristics of the study design were first extracted to a Microsoft Excel spreadsheet, such as the Exposure/Treatment groups and Control group(s); the number of participants per group; the quantitative measurement of depression; the intervention or treatment method, and the sequence strategy. Studies that included more than two groups were separated into subgroups according to the study design. Next, the quantitative information of the participants was extracted into the spreadsheet, such as the species, sex, weight, and age. The depression quantitative measurement values were then extracted, such as the questionnaire score or the time in the behavioural experiment. Afterwards, the study's results, including the microbiota diversity, beta diversity, and the abundance of specific taxonomy, were extracted. Lastly, the raw sequence data was extracted for multi-cohort analysis. Multi-cohort analysis was performed for all studies, which made their depression measures and microbiota data available. If the multi-cohort analysis was not possible, data was reported through a descriptive summary. Even if the paper did not describe the association between depression and microbiota in the text, the study was included in the multi-cohort analysis if depression quantitative data and microbiota data were available. Finally, eight studies were included in the multi-cohort analysis. Due to less than ten studies included in the multi-cohort meta-analysis, we do not test the funnel plot asymmetry.

The study design and participants' detail included in the multi-cohort analysis are as follows:

The sequence of Peter et al. (2018) cohort was downloaded from EBI under accession PRJNA386442. And the metadata was provided by the author. The metadata included the HADS of each participant. The dichotomous categorization of depressive/control was defined by HADS depression scores higher than 10.

The microbiota data in Strandwitz et al. (2019) was extracted from the American Gut Project (AGP)[12]. Sequence data of AGP was downloaded from EBI under accession ERP012803. Metadata was downloaded from the web portal (http://www.microbio.me/americangut)[12]. Due to the imbalanced sample sizes for the control and depressive group in the American Gut Project, we generated propensity scores for the samples in the subset healthy group, which were calculated based on the samples' reported age, BMI and sex. 386:386 depressive and controls were then selected by the *matchit* in *MatchIt* R package.

Sequence data from Hu et al. (2019) cohort was downloaded from EBI under the PRJEB23500. Since the samples ID in the sequence data of S. Hu(2019) did not match with the supplementary metadata in the original paper, and we did not get the responses from the authors to solve the issue, we directly used the sample ID in the sequence data to identify the group. The prefix “BD” was the BD group sample, and the prefix “H” was the health control. The suffix marked “-2” was the BD sample after treatment.

Sequence data from Borgo et al. (2017) was downloaded from EBI PRJNA375065. Metadata was downloaded from the Supplementary in this paper. The metadata provided the BDI score of each participant. The dichotomous categorization of depressive/control was defined by BDI depression scores higher than 14.

The Flemish Gut Flora Project (FGFP) dataset sequence data and metadata from Valles-Colomer et al. (2019) was downloaded from the European Genome-phenome Archive (EGA, https://www.ebi.ac.uk/ega/), accession no. EGAD00001004449. The data from FGFP cohort included 119 depressive and 914 non-depressive groups. Propensity scores were calculated based on the BMI, BSS score, age and sex. Then the *matchit* in *MatchIt* R package was used to find the matched control sample for the 119 depressive case sample.

The sequence and metadata of Schreiner et al. (2019) cohort were provided by the author. The HADS score and dichotomous categorization of depression overall score Higher_Than_7/ Lower_Than_8. We directly used this dichotomous categorization in the comparison analysis of the depressive group versus controls. For multi-cohort analysis, we only kept the samples with metadata.

The sequence and metadata of Vinberg et al. (2019) cohort were downloaded from Qiita (https://qiita.ucsd.edu/), Study ID: 12382. The metadata included the HDRS-17 score of each sample. We separated the sample into depressive group and control group by the HDRS-17 score cutoff 7.

The sequence and metadata of Kleiman et al. (2015) cohort were provided by the author. The samples included pre-treatment and post-treatment samples. We defined the pre-treatment sample as depressive and post-treatment as control.

Except for the study from Schreiner *et al* (2019), all the samples in the multi-cohort analysis were collected from human fecal. Due to the lack of the age, BMI and gender information, the unadjusted odds ratio in BD v.s. control group in Hu et al. (2019) and the crude result of Kleiman et al. (2015) were used to calculate the pooled adjusted odds ratio in the downstream meta-analysis.

Sequence quality control

Raw sequences and metadata from selected studies were retrieved from EBI or the corresponding author. A total of 1827 samples from eight cohorts were included in the meta-analysis. Cutadapt was used to remove the cohort-specific primer in the sequence with the default parameter. The reads were then processed by Qiime2.2019.7. Briefly, Fastq format reads were demultiplexed and visualized. Then the low-quality region at the end of reads was removed by DADA2 pipeline. Chimeric sequences were identified by

setting the –p-chimera-method as consensus. Cohort-specific parameters were shown in Supplementary Table 3. Picrust2 full pipeline in Qiime2 was used to predict and calculate the MetaCyc pathway abundances.

Taxonomy annotation and diversity calculation

After quality control, the feature table and sequences of each ASV were obtained by DADA2 and used for the taxonomic annotation. A pre-trained Naive Bayes classifier trained by the cohort-specific primer according to Silva 138 database and the q2-feature-classifier plugin was used. The ASV tables were rarefied according to cohort-specific depth. The rarefied ASV table was used to calculate the Observed OTUs, Chao1 indexes, Shannon diversity, Simpson diversity, Bray Curtis distance, Jensen–Shannon divergence, Weighted and Unweighted UniFrac distance. Taxonomy-level reads count profiles were collapsed by the ASV annotation result and rarefied ASV table.

Clusters of gut microbiota community

Dirichlet multinomial mixtures were used to classify different microbiota community types. Briefly, the reads count of genus-level from QIIME 2 Plugin 'taxa' were combined. ComBat_seq in *sva* package were used to adjust the batch effect. *dmn* function in *DirichletMultinomial* package was used to cluster the samples. The genus contribution to the cluster NMDS plot of samples was generated by the metaMDS in *vegan* package according to Bray Curtis distance. 1362 samples in seven cohorts (excluding the mucosa samples) were used in this analysis.

Meta-analysis of diversity

The median, first and third quartiles of Observed OTUs, Chao1 indexes, Shannon diversity, and Simpson diversity in depressive group and non-depressive group were obtained from each cohort. Then pooled difference of medians across groups was estimated by the quantile estimation method in *metamedian* package. The crude and adjusted odds ratio of high alpha diversity to depressive symptoms were pooled using the *metagen* in R *meta* package. Forest plots were ploted by the forest in *metafor* package.

PERMANOVA was conducted by adonis2 in *egan* package according to Bray Curtis distance, Jensen-Shannon divergence, Weighted and Unweighted UniFrac distance. The R-square value of each cohort was extracted from the PERMANOVA result. The 95% CI of R-square was calculated by bootstrap. A random-effects meta-analysis was used to calculate the pooled R-square. The PERMANOVA of all samples was calculated in 1634 samples with the complete metadata of age, sex and BMI.

Meta-analysis of taxonomy and pathway

Genus read counts were normalized into genus profiles by dividing the total read counts in one sample. *taxa.compare* in R *metamicrobiomeR* package was used to compare taxa relative abundance in each cohort. The estimates (log (odds ratio)) of each cohort were pooled by random-effects meta-analysis models by meta.taxa function. The output from meta.taxa consist of pooled estimates, standard errors, 95% CI, pooled p-values, and multiple testing adjusted pooled p-values for each bacterial taxon were

displayed by metatab.show and meta.niceplot function as a heatmap and forest plot. Taxa were only considered in the meta-analysis if it had been reported in more than 50% of cohorts.

The MetaCyc pathway abundances from the Picrust2 full pipeline were used to compare the difference between control and depressive group via *pathway.compare* in the R *metamicrobiomeR* package. The pooled estimates (log (odds ratio)) of MetaCyc pathway were calculated and visualized using the same method with taxonomy.

Support-vector machines classifier

The genus profile was used to build the SVM classifier. A total of 1827 samples were randomly separated into a training dataset or test dataset. A Learning Vector Quantization model was used to estimate the variable importance. Recursive Feature Elimination was used for automatic feature selection. The selected genera were used to build the SVM model by *rfe* function in the *caret* package. *tune.svm* in *caret* package was used to tune the parameters. The selected genus and parameters were used to build the final svm classifier.

Correlation and network of the genus

Correlations between genera were calculated using Spearman's rho rank correlation coefficients by *rcorr* in *Hmisc* package. We calculated the correlation separately for each depressive and control sample group, in each of the eight cohorts, respectively. The pooled correlation was estimated by metacor in *meta* package. Correlation with adjusted q value < 0.05 and correlation rho value > 0.4 or <-0.4 were used in the network analysis and visualized by Cytoscape.

Results

Study search, selection and summary of the published literature

We followed the PRISMA guideline[13] to systematically search and filter studies. Firstly, we combined literature from PubMed, Scope and Web of Science and filtered it according to our eligibility criteria (see Supplementary Methods). From the 491 unique records identified, 46 reports were excluded after title and abstract screening, and another 416 articles were excluded after full-text screening. Of the remaining studies, 29 reported sequence data and depression-related phenotypes (Supplementary Fig. 1A), but only 24 studies directly reported the association between gut microbiota and clinical observations of depression (Supplementary Table 1). These 24 studies were included in our systematic review. Their study designs included case-control studies (18), cohort studies (3), quasi-experiments (2) and randomized-controlled trials (RCT) (1). Sample sizes of these studies ranged from 20 to over 2000. Most studies were from Europe (10) and Asia (9). The 16S V3-V4 (9) and V1-V2 (3) regions were sequenced. Hamilton's Depression Scale (9) and Beck's Depression Inventory (8) were the most frequently used tools for assessing depression severity. Participants who were clinically diagnosed with depression or scored a

higher depression score than healthy controls were considered as depressive cases. Participants who did not score as depressive served as control groups.

We summarised the 24 studies' original results in terms of their alpha diversity and taxonomic variations of gut microbiota between the depressive and non-depressive groups (Supplementary Table 2, Supplementary Fig. 2–3). Thirteen studies reported an absence of significant differences in alpha diversity between the depressive group and controls. Eight studies reported a significant decrease in the alpha diversity in depressive groups. However, an increase in the alpha diversity of gut microbiota in depressive groups versus healthy controls was reported by two studies (Supplementary Fig. 2, Supplementary Table 2). As for beta-diversity analysis, ten studies reported a distinct gut microbial community in depressive groups compared to healthy controls (Supplementary Table 2). In terms of relative abundances of microbial taxa, depressive groups had significantly reduced *Dialister*, *Coprococcus*, *Clostridia* and *Chitinophagaceae*. In contrast, the relative abundance of *Parabacteroides*, *Eggerthella*, *Bifidobacterium*, *Streptococcus*, *Desulfovibrio*, *Selenomonas*, *Rothia*, *Holdemania*, Fusobacteriaceae, *Escherichia*, *Enterobacter*, Desulfovibrionaceae, *Acidaminococcus* showed a consistent enrichment in the depressive group (Supplementary Fig. 3).

The association between gut microbial diversity and depression across cohorts

Among the 29 studies reporting sequencing data and depression phenotypes, only eight studies provided 16S rRNA raw sequencing data, depression scores or depressive phenotypes. We performed a standardized bioinformatic pipeline on these studies to retrieve the taxonomic profiles and metabolic pathways (Method, Supplementary Table 3) and a total of 1827 samples from references 14–21[14–21] were included in the present multi-cohort study (Supplementary Fig. 1B).

We calculated the pooled estimated median difference in alpha diversity between depressive (n = 695) and control groups (n = 1152) by deducting the alpha diversity index of the depressive group from the index of the control group. We calculated the median differences with different indexes, including Observed Operational Taxonomical Units (OTUs)(2.44), Chao1 indexes (-0.52), Shannon diversity (0.14), and Simpson diversity (0.005; Supplementary Fig. 4). None of the indices showed a significant difference between depressive and control groups.

The data for two studies were evaluated for sensitivity analysis: Schreiner *et al.* (2019) extracted sequencing data from intestinal samples. Hence, all data from this cohort were excluded from analysis. Meanwhile, Hu *et al.* extracted data from controls, and patients before and after Bipolar Disorder (BD) treatment. The sequencing data from participants post-treatment may induce heterogeneities. Hence, this portion of data was excluded. The pooled estimate values from the remaining seven cohorts (n = 615 and 725 in depressive and control groups respectively, Supplementary Fig. 4) suggest increased alpha diversity in controls relative to the depressed group, albeit without statistical significance. To further minimize confounding effects, the adjusted odds ratios with 95% CI from these seven cohorts were pooled. There was a greater heterogeneity in richness indexes (Chao1 index and observed OTU)

compared to evenness indexes (Simpson and Shannon). Nevertheless, the alpha diversity did not significantly differ between depressive and control groups. However, the pooled odds ratios in all four indexes were less than 1, indicating a negative relationship between the alpha diversity of gut microbiota and depressive symptoms after adjusting for age, sex and Body Mass Index (BMI), without reaching statistical significance(Fig. 1).

The comparison of beta diversity between depressive and control groups in each study was conducted by PERMANOVA based on Bray Curtis distance, Jensen–Shannon divergence, Weighted and Unweighted UniFrac distance (Supplementary Fig. 5). In the study by Hu *et al.* (2019), the gut microbial communities were significantly different between the depressive group and controls. Similarly, Schreiner *et al.* (2019) also reported significant differences in terms of Bray Curtis distance, Jensen–Shannon divergence, and Unweighted UniFrac distance. However, the extent of variance in the microbial community that can be explained by depression (R square) was low. While the highest R^2-value was 7.6%, which was calculated by the Jensen–Shannon divergence in the Hu *et al.* (2019) cohort, the pooled estimates of R^2-values in these eight cohorts were only 1% (Supplementary Fig. 6). PERMANOVA was also carried out in all samples with the relative metadata in eight cohorts (n = 1634, Supplementary Table 4). Depression score, age, gender, BMI and cohort all exhibited significant effects on the gut microbiota community at the genus level. Only 1.34% of the total variation, however, was explained by depression and this value was lower than that of gender and cohort (13.42% and 8.18%, respectively, Supplementary Table 4).

We then calculated the dispersion of gut microbiota communities in depressive and controls groups(Supplementary Fig. 5) and found that it was significantly higher in the depressive group in the cohorts by Peter *et al.* (2018), Hu *et al.* (2019) and Schreiner *et al.* (2019). However, results by Kleiman *et al.* (2015) showed the opposite.

Microbial taxa and metabolic functions associated with depression

A meta-analysis with random-effects model was used to pool estimates from eight cohorts and investigate the effect of depressive phenotypes on gut microbiota composition across different populations. As mentioned earlier, sensitivity analysis was also conducted by removing the Schreiner *et al.* (2019) cohort, since the different sample types (mucosal vs faecal samples) may induce biases. The relative abundance of *Firmicutes* was lower in the depressive group, even after sensitivity analysis and adjusting for age, sex and BMI (Fig. 2). By contrast, the relative abundance of *Bacteroidetes* was elevated in the depressive group, even after sensitivity and adjustment analyses (p = 0.057 and 0.07, respectively). The relative abundance of *Parabacteroides*, *Barnesiella* and *Bacteroides* were significantly elevated in the depressive group in the crude model (OR [95% CI]: 1.10 [1.02–1.20], 1.13 [1.02–1.25], 1.20 [1.02–1.42], respectively) at the genus level, despite adjustment for confounding factors. At the species level, the relative abundance of *Parabacteroides distasonis*, *Alistipes inops* and *Bacteroides vulgatus* were significantly enriched in the depressive group in all analyses (*P. distasonis*: p-value = 0.054 in the adjusted

model), and the *Oscillospiraceae* UCG 002 sp, Clostridia UCG 014 sp and *Desulfovibrio* sp. were enriched in control group in the crude model.

After adjusting for confounding factors, we identified several differential gut microbiota taxa between depressive and control groups. For example, *Dialister*, *Bacteroides plebeius* and *Desulfovibrio sp.* were elevated in the control group. Yet, *Megasphaera*, *Intestinimonas*, *Hungatella*, *Bacteroides massiliensis, Christensenellaceae R7 group sp.* and *Sutterella sp.* were increased in depressive groups in the adjusted model, but only after excluding mucosa samples (Fig. 2).

The PICRUSt2 pipeline was used to predict MetaCyc pathways based on 16S data. We compared the relative abundances of MetaCyc pathways between groups. We performed the comparison with and without adjusting confounders. In total, 64 MetaCyc pathways were significantly different between depressive and control groups (Supplementary Fig. 7). There were more pathways which showed significant enrichments in the control group (42/64), even though the odds ratios were small (absolute value for the log of an odds ratio was < 0.1), indicating significant, albeit small, differences. Numerous pathways were robustly enriched in the depressive group, including L-histidine degradation I. By contrast, the L-isoleucine biosynthesis pathways, I, II, III, IV, and the L-isoleucine biosynthesis I super pathway were all consistently elevated in controls.

Gut microbiota-based classification of depression

To assess whether the depressive and control group could be differentiated by gut microbiota, we built a support vector machine (SVM) model based on gut bacteria genera. The top 30 bacterial genera were selected by their importance scores (Fig. 3A-B; Methods). Bacterial genera such as *Parabacteroides* and *Hungatella* that best differentiate between depressive samples and controls also overlapped with markers identified in the taxonomic meta-analysis.

All samples were separated into training and test datasets with a split ratio of 80:20. The area under curve (AUC) was 0.90 and 0.65 in training and test datasets, respectively, as obtained by the SVM model (Fig. 3C-D). The average AUC of the same model used in the single cohort reached 0.78 (Supplementary Fig. 8). We further evaluated the classification power of a set of predictors including bacteria taxa together with age, sex and BMI. For this, we included 1308 samples in the training dataset and 326 samples in the test dataset with complete metadata. The AUC of the combined model increased to 0.95 in the training dataset, with mild predictive power (AUC: 0.64) in the test dataset (Fig. 3C-D), which is higher than the model based on metadata alone (0.53 and 0.54 with training and test datasets, respectively).

Using the model established with microbial data and metadata, we calculated the correlation between the depressive scores predicted by SVM model and reported instances of depression scores obtained from reference14,17, 19 and 20[14, 17, 19, 20]. Correlations were significant and relatively high in the Peter *et al.* (2018), Borgo *et al.* (2017) and Vinberg *et al.* (2019) cohorts (0.68, 0.72 and 0.60, Supplementary

Fig. 9). However, we did not obtain a high correlation value in the Schreiner *et al.* (2019) cohort, which used different sample types.

Microbiota interaction networks in depression

To explore the gut microbiota ecology in depressive and control groups, we calculated pooled correlations between genera from the main analysis, including eight cohorts, and sensitivity analysis, including seven cohorts. Correlations with a p-value less than 0.05 were included in downstream analyses. The correlation in depressive group was stronger than that of controls (p-value < 0.01) for 183 correlations, with a *rho* value higher than 0.4 or less than − 0.4. Only 13 correlations showed negative relationships (Fig. 4). Among the control group, 3 of 106 correlations were negative. Seventy-three correlations were detected in both depressive and control interaction networks. The depressive network had a higher network centralization compared with the control network (0.25 and 0.35, respectively).

Three depressive-enriched markers, *Barnesiella*, *Bacteroides* and *Parabacteroides*, and one control-enriched marker, *Dialister*, in previous taxonomic meta-analyses were found in the network of control group, whereas five were found in the depressive network. None of these markers was hub nodes in these two networks. *Barnesiella* showed a consistent correlation with *Alistipes* in both networks, while *Alistipes* showed a consistent correlation with Oscillospiraceae UCG 005, Oscillospiraceae UCG 002 and Christensenellaceae_R.7_group, the hub node of these two networks.

Biomarkers used in the SVM model could also be found in the two interaction networks. Thus, *Alistipes*, *Fusicatenibacter*, *Phascolarctobacterium* and *Romboutsia* are markers that show significant correlations in the depressive and control networks. However, the interactions of these markers with other genera were different. *Alistipes* only showed a significant correlation with *Odoribacter* in the depressive group. *Fusicatenibacter* positively correlated with *Faecalibacterium* in the depressive group and *Agathobacter* in the control group. Eight additional biomarkers in the SVM model were unique in depression, whereas two biomarkers were unique in the control group.

Gut Microbiota Cluster Associated with Depressive

Due to the different ecology networks of gut microbiota in depressive and control groups, the gut microbiota cluster was also conducted using Dirichlet Multinomial Mixtures (DMM). Given that the sampling locations varied in the study by Schreiner *et al.* (2019), we did not include these samples for community typing analysis. All 1362 samples were separated into two clusters (Fig. 5A-B). Cluster 1 showed a significantly lower likelihood of having depressive symptoms compared with cluster 2 (Supplementary Table 5). Compared with cluster 1, cluster 2 had a 32% (95% CI: 1.06–1.64) higher likelihood of depressive symptoms. After adjusting for age, sex and BMI, the odds ratio remained at 1.30 (1.02–1.65). The top ten gut taxa that contributed to clustering demonstrated significant intercluster differences. Cluster 1 presented a lower abundance of *Escherichia-Shigella*, and a higher abundance of *Faecalibacterium*, Oscillospiraceae UCG 002, *Ruminococcus* and Christensenellaceae R.7 group (Fig. 5C).

Discussion

This study is the first comprehensive meta-analysis of the gut microbial 16S rRNA sequencing data of participants with depressive symptoms. Eight different cohorts with 16s rRNA sequencing data were systemically screened from published journals and combined for analysis. There was no significant difference in alpha diversity between the depressive-associated microbiota and microbiota of healthy controls. But the overall community of depressive-associated microbiota was significantly different from that of health controls in terms of the Bray Curtis distance. Regarding significantly different taxonomic and functional biomarkers between depressive and control samples, consistent biomarkers across eight cohorts was identified. Thus, we established a classifier with an AUC of 0.95 in training set and 0.64 in testing set from the combined 16s sequencing data of eight cohorts, using a group of microbes that differentiate the gut microbiota between samples of depressive and healthy individuals. An interaction network analysis was conducted to further characterize differences in gut microbiota ecology between depressive and control groups. A gut microbiota cluster with a reduced risk of depressive symptoms was represented by lower abundance of *Escherichia-Shigella* and a higher abundance of *Faecalibacterium*, Oscillospiraceae UCG 002, *Ruminococcus* and Christensenellaceae R.7 group. These findings indicated that the overall ecology of gut microbiota and interaction between microbial communities might be more important for the development of depression, rather than a single taxonomy player. In summary, the findings of the present study emphasize the need for the holistic monitoring of gut microbiota to assist in the diagnosis of depression.

The present study does not show a significant alteration in alpha diversity of the gut microbiota among depressive groups. However, we identified significant changes in beta diversity and the relative abundance of certain taxa after combining results from different cohorts. These results corroborate findings by recent systematic reviews of gut microbiota associated with psychiatric disorders[10, 22]. Moreover, we observed a significant enrichment in the abundance of *Firmicutes*, particularly the abundance of *Dialister*, in the pooled result of the adjusted model. *Dialister*, which is considered to be positively associated with quality of life[18], was consistently depleted in depressive groups in four studies (Supplementary Fig. 3). Acetate, lactate and propionate, being the metabolic end-products produced by *Dialister*[23, 24], could increase the ability to resist stress and exert antidepressant-like effects by modulating histone acetylation and deacetylases[25, 26]. At the species level, *B. plebeius* was reported to be significantly enriched in the control group by our meta-analysis. Glutamate can be synthesized by *B. plebeius* PB-SLKZP[27, 28], acting as a primary precursor of γ-aminobutyric acid (GABA), which plays a prominent role in stress control by the brain, improving the resilience of individuals towards depression[29].

The relative abundance of Bacteroidetes was enriched in the depressive group compared to controls. *Parabacteroides*, *Barnesiella* and *Bacteroides* are members of the Bacteroidetes phylum, which are also reported to be enriched in depression (Supplementary Fig. 3). *Parabacteroides* supplementation was reported to interact with tryptophan (Trp) metabolism pathways in the mouse hippocampus, ameliorating the toxic, depression-related production of kynurenine (Kyn) and the associated metabolites[30]. Despite the proposed beneficial role of *Parabacteroides* against depression, the enrichment of *Parabacteroides* in microbiota of depressed individuals has been consistently reported by multiple studies (Supplementary

Fig. 3), and can induce depressive-like behaviour in mice[31]. These inconsistencies highlight the importance of standardizing protocols for meta-analysis, as we have done in the current review, and to achieve a higher taxonomic resolution to establish a more precise interaction between microbiota and depression.

At the species level, *A. inops*, and *B. vulgatus* were enriched in the depressive group. *A. inops* is an indole-positive bacterium[32], which produces indole to decrease serotonin availability. Since it disrupts the gut-brain axis, *A. inops* may be associated with the onset of depression[33]. *B. vulgatus* also promotes NF-κB expression and the downstream pro-inflammatory signalling cascade in intestinal epithelial cells[34] to induce depressive-like phenotypes[35]. We found that *Sutterella*, a Betaproteobacteria belonging to the Proteobacteria, was enriched in depression. In contrast, two other Proteobacteria, *Morganella* and *Klebsiella*, which are both Gammaproteobacteria, were not different in our analysis, although they have been postulated to have causal effects on MDD in a large cohort study including 5,959 individuals[36].

GABA deficiency may contribute to depressive disorders[29]. In our meta-analysis of this pathway, we identified consistent results according to 16S rRNA predicted pathways. L-glutamate is the primary precursor of GABA. In our study, several L-glutamate production pathways (UDP-N-acetyl-D-glucosamine biosynthesis I; UMP biosynthesis I; L-arginine biosynthesis I (via L-ornithine)) were enriched in the control group, whereas the pathway related to L-glutamine degradation, dTDP-N-acetylthomosamine biosynthesis, was enriched in the depressive group. Remarkably, the L-isoleucine biosynthesis I, II, III, IV, pathways and the L-isoleucine biosynthesis I superpathway were clustered in the control group. In keeping with previous studies that observed an inverse association between isoleucine intake and odds of depression and anxiety[37], our meta-analysis also reveal a reduction of L-isoleucine biosynthesis in depressive samples. Overall, analysis of biochemical pathways identified more markers associated with depression compared with taxonomy, which may indicate convergent functional changes regardless of the inconsistent taxonomic changes in depression.

Even though consistent taxonomic markers can be found across the cohorts, more markers need to be used to establish the classifier to discriminate between depressive and control groups. The classifier, which combined the selected microbiota and metadata, had an AUC of 0.86 in the overall dataset. The AUC was higher than 0.8 in most cohorts after excluding BD patients[16] and mucosa samples[19]. The predicted score also shows a high correlation with measured depression scores, indicating the potential of this classifier in predicting the initial phase of depressive symptoms or mild depressive patients. The different interaction networks in depressive and control groups further suggest that rather than single taxa, the ecology of the overall gut microbiota might be more dominantly involved in the development of depression, especially two of the hub nodes, *Oscillospiraceae* UCG 002 and *Christensenellaceae* R7 group, which were also associated with microbial cluster representing low risk of depression. Furthermore, the reduced abundance of an inflammatory taxon, *Escherichia-Shigella*, and the higher abundance of butyrate-producing bacteria *Faecalibacterium*, Oscillospiraceae UCG 002, *Ruminococcus* and Christensenellaceae R.7 were associated with a reduced risk of developing depressive symptoms. The higher abundance of *Oscillospiraceae* UCG 002, *Christensenellaceae* R.7 and lower abundance of

Parabacteroides in the low risk cluster were consistent with the meta-analysis result. The contradiction and heterogeneity between previous studies may be partially explained by uneven sample sizes between the two clusters during comparison. It might also influence the response and non-response to anti-antidepressants and probiotics. The reduced risk identified in the cluster analysis provides insight into the potential preventive function of gut microbiota in the development of depression.

Our study is the first multi-cohort meta-analysis to aggregate microbiota sequence data from diverse depressive populations and use a uniform analytical method to identify gut microbiota characteristics in depressive participants across cohorts and across different analytical approaches. We controlled common confounding factors such as age, sex and BMI to reduce bias caused by statistical analyses. However, other important confounders such as the use of antidepressants[18], frequency of alcohol consumption and bowel movement quality[38] were not considered due to lack of data. Another limitation is that the number of cohorts was much less than the systematic screening result. A total of 21 of the 29 studies could not be included in the multi-cohort analysis due to lack of raw sequence data. Hence, further multi-cohort studies with more high-quality sequences will improve the accuracy of microbiota-based classification and provide a deeper understanding of microbiota function in depression.

In conclusion, the standardization of data analysis using raw 16s rRNA sequencing data collected from available literature revealed an absence of significant changes in alpha diversity of gut microbiota between participants with depressive symptoms and healthy controls. However, the current meta-analysis demonstrates significant changes in beta-diversity of gut microbiota between depressive and control groups. This study has also successfully identified consistent changes of certain bacterial taxa associated with depressive symptoms, as well as metabolic pathways that contribute to interactions with host physiology. Universal community and ecological shifts related to depressive symptoms have also been discovered in the depressive group, providing directions for the potential inclusion of gut microbiota analysis in depression scoring. The classifier established in this study had AUC values higher than 0.78 in cohorts, further indicating that the gut microbiota could be used as a practical predictive tool and, possibly, as preventive and treatment option for depression.

Declarations

Data availability

Full documentation of metadata and raw sequence were downloaded from the original paper (Supplementary Methods). The main scripts used in the current study are available on GitHub (https://github.com/suishal/Multi-cohort_depressive).

Competing Interests

The authors declare that they have no conflict of interests.

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Author Contributions

S.L and Z.S searched, selected and extracted the data from published papers. S.L analyzed data and drafted the manuscript. Z.S, S.Z, X.Z, J.Y, R.B and H.T commented on the study and revised the manuscript. H.T supervised the study. All authors read and approved the final manuscript.

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Figures

Figure 1

Association of alpha diversity indices with depression. The pooled odds ratio was calculated by pooling the adjusted odds ratio of alpha diversity values associated with depression in seven cohorts (n=1342). Odds ratio values that are higher than 1 indicate a higher odds ratio for the depressive group compared to healthy controls.

Figure 2

Differential microbiota biomarkers associated with depression. The adjusted model shown at the (A): species level; (**B**): genus level; (**C**): phylum level. The crude model shown at the (**D**): species level; (**E**): genus level; (**F**): phylum level. The colour in the heatmap indicates the log(OR) in each cohort. White indicates missing values. * : p-value <=0.05, ** : p-value <= 0.0001. The line in the forest plot indicates the 95%CI of pooled log(OR). Red indicates p-value <=0.05. Triangles indicate that the false discovery rate (FDR) adjusted p-values < 0.1. Genera and species from the same phyla are labelled with the same colour.

Figure 3

Microbiota-based classification of depression. (**A**) Line plot showing classifier accuracy against the number of features. The red dot indicates the highest accuracy (n=30). (**B**) Top 30 most important genera identified after automatic feature selection via Recursive Feature Elimination. The heatmap on the left indicates whether the genus was enriched in depressive or control samples. *indicates the p-value <0.05. The heatmap in the middle indicates the mean abundance of the genus in the control and depressive group. The right lollipop chart shows the importance of each genus. G: "Un" indicates unclassified genus in the listed family. G: "Uc" indicates uncultured genus in the listed family. (**C**): the ROC curve in the training set. (**D**): the ROC curve in the testing set. The black curve and AUC are the results obtained using microbiota data and metadata (age, BMI and sex). The blue curve shows the results obtained using microbiota data only.

Figure 4

Microbiota occurrence networks for between control (**A**) **and depression** (**B**) **at the genus level.** Only correlations with adjusted q value <0.05 and rho value >0.4 or <-0.4 were used in the network analysis. The node colour indicates the phylum of the node. The size of the nodes indicates the number of connected genera. Orange lines indicate a positive correlation; blue lines indicate a negative correlation. Solid lines indicate a correlation found in the pooled estimate calculated from both the main analysis (eight cohorts) and the sensitivity analysis (seven cohorts). Line width indicates the size of the correlation. The dashed lines indicate the correlation found only in the main analysis.

Figure 5

Microbiota clusters associated with the risk of depression. (**A**) Non-metric multidimensional scaling (NMDS) ordination of samples. Stress = 0.17. Different colours indicate samples belonging to different clusters. (**B**) Odds ratios for depressive symptoms among gut microbiota clusters. The blue line indicates the crude model; the red lines indicate the adjusted model. (**C**) Top ten taxonomic contributions to Dirichlet components in each cluster. Differences in abundance between clusters were analyzed by the Wilcoxon test. ns: not significant. ***:p <0.001. ****: p <0.0001.

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