Gut microbiome-wide association study of depression

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Article

Keywords: gut microbiome, depression, pathogenesis

Posted Date: June 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-570388/v1

Version of Record: A version of this preprint was published at Nature Communications on December 6th, 2022. See the published version at https://doi.org/10.1038/s41467-022-34502-3.

Abstract

Depression is one of the most poorly understood diseases due to its elusive pathogenesis. There is an urgency to identify molecular and biological mechanisms underlying depression and the gut microbiome is a novel area of interest. In this study we investigated the relation of fecal microbiome diversity and composition with depression in 1,054 from the Rotterdam Study cohort and validated these findings in the Amsterdam HELIUS cohort in 1,539 subjects. Using supervised and unsupervised machine learning approaches, we identified and replicated the association of several microbial genera. We confirm the association of genus *Eggerthella, Subdoligranulum, Coprococcus* and family *Ruminococcaceae* and identify novel bacteria including *Sellimonas, Lachnoclostridium, Hungatella, Ruminococcaceae (UCG002,UCG003 and UCG005), LachnospiraceaeUCG001, Eubacterium ventriosum* and *Ruminococcusgauvreauiigroup* associated with depression. These bacteria are known to be involved in the synthesis of glutamate, butyrate, serotonin and gamma amino butyric acid (GABA), which are key neurotransmitters for depression. Our study suggests the gut microbiome composition may play a key role in depression.

Introduction

Depression is one of the most common mental disorders experienced worldwide with an average lifetime prevalence of 11–15% [1]. The prevalence has doubled and, in some countries, even tripled during the COVID-19 pandemic [2]. Yet, depression is also one of the most common and poorly understood diseases courtesy of its elusive pathogenesis. Treatment options are sub-optimal with most antidepressants performing only marginally better than placebo[3, 4] with additional costs of having side effects ranging from minor cognitive complaints to even suicide[5]. The low to moderate heritability [6] and the small effects of genetic variants (odds ratio < 1.05) identified in large genome-wide association studies (GWAS) of depression [7] indicate the need to search for determinant beyond genetics.

Evidence is accumulating that gut microbiota may influence brain activity and behavior via neural and humoral pathways [8, 9]. It has been speculated that the gut microbiome may have translational applications in the treatment of neuropsychiatric disorders [10–12] including depression, which is responsible for significant disability worldwide [13]. Several animal studies suggest that gut microbiota might have impact on the neurobiological features of depression [14–22]. Fecal microbiota transplantation of either stressed or obese animals to control animals showed significant alteration of anxiety [23]. Data in humans are scarce. Two studies in humans showed that pre- and probiotic consumption positively affects mood and anxiety [24, 25]. There have been very few studies exploring the relationship between gut microbiome and depression in humans [26] and these studies are based on very small samples (< 60 cases), lacking statistical power to detect robust association. The most recent and largest study including 121 cases reported depletion of butyrate producing bacteria (*Coprococcus* and *Dialister*) in individuals with depression [27]. However, these studies did not adjust for confounders including psychiatric and other medication [26] which may modify the gut microbiome [28]. Overall the results of existing studies are conflicting with little overlap asking for larger and more carefully designed studies. Here, we study the effect of gut microbiome diversity and composition on depression scores in 1,133 individuals from the Rotterdam Study. The results were replicated in an independent cohort (HELIUS-study, N = 1,539). Finally, we performed Mendelian Randomization (MR) to elucidate causal relationships between the identified microbiota and major depression.

Materials And Methods

Study population

The discovery cohorts include 1,054 participants from the Rotterdam Study who were not using anti-depressants at the time assessment. The Rotterdam Study is a population-based cohort study from the well-defined Ommoord district within Rotterdam, The Netherlands. It is designed to investigate occurrence and determinants of diseases in the elderly [29]. Initially, the RS included 7983 participants in 1990 who underwent an at-home interview, extensive physical examination at baseline and during follow-up examinations that occur every 3–4 years (RS-I). The RS was extended with two more cohorts in 2000 (RS-II) and 2005 (RS-III) and contains a total of 14,926 participants. In this study we used the data of individuals from the second follow up of the third Rotterdam Study cohort (RS-III-2) as these individuals were profiled for the gut microbiome. The RS is approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The RS was entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

The replication cohort included 1539 participants from the Healthy Life in an Urban Setting (HELIUS) cohort. The HELIUS cohort is a multiethnic cohort consisting of individuals of Dutch, Surinamese, Ghanaian, Turkish and Moroccan origin from Amsterdam. People in the age range of 18–70 years were randomly sampled, stratified by ethnic origin, through the municipality register of Amsterdam. This register contains data on country of birth of citizens and of their parents, thus allowing for sampling based on the widely accepted Dutch standard indicator for ethnic origin [30]. The Dutch sample includes people who were born in the Netherlands and whose parents were born in the Netherlands. The current

study used data from Dutch samples only. The Medical Ethics Committee of the Amsterdam UMC, location AMC approved the study protocols. Written informed consent was obtained from all participants.

Fecal sample collection and microbiome profiling

Detailed description on how the gut microbiome composition was generated at RS-III-2 (2012-13) and in the HELIUS cohort are described elsewhere [31, 32]. Briefly, in RS, participants were instructed to collect a stool sample at their home in sterile tubes and to send the sample by regular mail to the research location of Erasmus Medical Center (EMC), Rotterdam, the Netherlands. Upon arrival at EMC samples were checked and stored at -20°C. Subsequently, an automated stool DNA isolation kit (Diasorin, Saluggia, Italy) was used to isolate bacterial DNA from approximately 300 mg stool aliquot using a bead beating step. The V3 and V4 hypervariable regions of the bacterial 16S rRNA gene were amplified and sequenced on an Illumina MiSeq platform.

Participants from the HELIUS-study were given a stool collection tube and requested to collect a stool sample. Participants brought the samples to the research location within 6 hours after collection and if not possible kept in their freezer overnight and brought to the research location the next morning. At the research location, the samples were temporarily stored at − 20°C until daily transportation to the Amsterdam Medical Center (AMC), Amsterdam, the Netherlands, where the samples were checked and stored at − 80°C. Total genomic DNA was extracted from a 150 mg aliquot using a repeated bead beating method. The V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform.

For both cohorts after initial quality filtering and rarefaction analysis the reads were subsampled at 10,000 reads per sample. Taxonomy was assigned to the obtained reads using RDP classifier (version 2.12) and SILVA database (version 128). Alpha diversity indices such as species richness, Shannon index and Inverse Simpson were calculated at the genus-level. We calculated Bray-Curtis distance based on absolute abundance of microbial communities at genus level to measure beta-diversity. Taxa present in in less than 3% of the sample size (each cohort separately) and taxa with read count less than 0.005% were excluded. Taxa abundances (absolute counts) were than log transformed (to the absolute values 1 was added before log-transformation).

Depression assessment

In RS depressive symptoms were assessed using the 20-item version of the Center for Epidemiological Studies-Depression (CES-D) scale [33]. CESD is a self-report measure of symptoms experienced during the prior week. It has been shown to be relatively stable over time and covers the major dimensions of depression including depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite and sleep disturbance [34]. The total score ranges from 0 to 60, with higher scores indicating a greater burden of depressive symptoms. The CESD detects current MDD cases with high sensitivity and specificity. We used the depression assessment from RS-III-2 (the same time as the collection of the feces).

For participants of the HELIUS cohort, depression was assessed using the Patient Health Questionnaire (PHQ-9) design [35]. PHQ-9 scores each of the DSM-IV criteria as “0” (not at all) to “3” (nearly every day). The total score ranges from 0 to 21, with higher scores indicating severity of depression. A PHQ-9 score of ≥ 10 has a sensitivity and specificity of 88% to detect major depression. Cases of major depression were defined as those scoring ≥ 10 on PHQ9 scale. Individuals with ethnic background other than Europeans and individuals using antidepressants were excluded.

Statistical analyses

Microbiome association analysis

To test the association of depressive symptom scores with alpha diversity and individual taxa we used linear regression models using depression scores as the outcome and alpha diversity and taxa as independent variables adjusting for several covariates including sex, age, alcohol use, body mass index (BMI), smoking, medication use (proton pump inhibitors (PPI), metformin, lipid-lowering and antibiotics) and technical covariates including time in mail and batch (in case of RS cohort). Association of the depression scores with microbiome beta-diversity was performed using permutation analysis of variance (PERMANOVA) in R-package “vegan” using the same model as described above.

Results from the discovery and replication cohorts were combined in a meta-analysis using METAL software [36]. Since the depressive symptoms assessment scales were different in the discovery and replication cohorts, we used sample-size weighted meta-analysis to combine the results. Adjustment for multiple testing was performed using false discovery rate (FDR).

Further, we used the random forest regression algorithm with RS stool microbiome profiles as predictors and depression scores as response for the training. In the same model we used HELIUS stool microbiome profiles and depression scores as predictors and response, respectively, as the test data. In the model we used 500 trees (ntree = 500) and 100 number of variables randomly sampled as candidates at each split (mtry = 100). In addition, we set the number of times the out of bag data is permuted per tree for assessing variable importance to 100 (nPerm = 100).

The increase in mean square error (%IncMSE) was calculated and plotted for each taxon. %IncMSE provides the most robust and informative value since this is the increase in MSE of prediction and is estimated with out of bag cross validation.

Mendelian Randomization (MR) analysis

To ascertain causal links between the identified microbiota and major depressive disorder (MDD) we performed MR analysis using the results of the largest genome-wide association studies of both microbiome and major depression[7, 37]. For major depression we used 87 genome-wide significant single nucleotide polymorphisms (SNPs) reported by Howard et al. 2019 [7] as instruments (**Supplementary Table 1**). For microbiome there were none to a very few SNPs that were genome-wide significantly associated with the identified microbiota, so we used SNPs (top SNP per locus) with a p-value < 10^{-05} as instruments (**Supplementary Table 2**). For the analysis we used the 'mr_allmethods' option of the library "MendelianRandomization" of the R software [38]. This method reports results from the median method (simple, weighted and penalized), Inverse variance weighted (IVW) and Egger methods (penalized, robust and penalized & robust).

We further examined the microbiome-wide association of each of the 87 SNPs associated with depression using the microbiome GWAS summary statistics from Kurilshikov, A. *et al*.[37] to identify the gut microbiota associated with these SNPs. Finally, we tested the association of the genetic risk score combining the summary level data of the 87 SNPs for each microbiota in an unweighted genetic risk score using inverse-weighted method in the 'rmeta' package of R software.

Results

Microbiome association analysis

The cohort characteristics are provided in Table 1. After exclusion of individuals using antidepressants and non-European subjects, 1,054 samples from RS and 1,539 samples from the HELIUS-study were included in the analyses (Table 1). The resulting microbiome data consisted of 17 phyla (for both cohorts), 33 classes for RS and 36 classes for HELIUS, 59 orders in RS and 61 orders for HELIUS, 116 families for RS and 108 families for HELIUS, and 439 genera for RS and 418 genera for HELIUS. In both cohorts, microbiome was dominated by phyla Firmicutes (77% in RS and 70% in HELIUS), Bacteroidetes (13% in RS and 21% in HELIUS), Actinobacteria (0.42% in RS and 0.42% in HELIUS) and Proteobacteria (0.48% in RS and 0.22% in HELIUS).

Table 1
Descriptive statistics of the study populations

	RS	HELIUS
Age: mean(± SD; range)	56(± 5.9; 45–87)	51(± 12.8; 19–71)
Sex (female%)	56%	49%
BMI: mean(± SD; range)	27(± 4.4; 16–51)	26(± 4.4; 16–53)
Smoking (current, ever, never)	(137, 533, 384)	(305, 664, 568)
Antidepressants (Yes)	79	66
Depression Score mean(± SD; range)	4.7(± 6.2; 0–49)	3(± 3.6; 0–24)
Descriptive statistics of the Rotterdam Study (N = 1,504) and HELIUS (N = 1,539) cohorts.		

Alpha diversity was negatively associated with depressive symptoms in both RS (Shannon index; beta = -1.57, p-value = 1.5×10^{-03}) and HELIUS cohorts (Shannon index; beta = -0.64, p-value = 2.84×10^{-02}). Beta diversity showed significant association with depressive symptoms in RS (Permanova; R^2 = 0.003, p-value = 0.001) but not in the HELIUS cohort (R^2 = 0.0005, p-value = 0.51).

At taxanomic level, 24 genera, three microbial families, one class, two orders and a phylum were significantly (false disccovery rate (FDR) < 5%) associated with depressive symptoms in the Rotterdam Study (Table 2, **Supplementary Table 3**). We replicated these results in the HELIUS cohort for 12 genera which were associated with depressive symptoms scores in the same direction (Table 2). These include *Sellimonas, Eggerthella, Ruminococcaceae (UCG002, UCG003, UCG005), Coprococcus3, Lachnoclostridium, Hungatella, LachnospiraceaeUCG001, Ruminococcusgauvreauiigroup, Eubacterium ventriosum, Subdoligranulum* and *Ruminococcus*. *Sellimonas, Eggerthella, Lachnoclostridium and Hungatella*. Of the three microbial families significantly associated with depression in RS, we replicated one in the HELIUS cohort: family Ruminococcaceae. The direction of association was consistent for all associated taxa across both cohorts and the meta-analysis of results from both cohorts improved association p-values (Table 2).

Table 2
Microbiota significantly associated with depression

	Rotterdam Study (N = 1054)				HELIUS (N = 1539)			Meta-analysis		
Taxon	Beta	Se	p	fdr	Beta	Se	P-value	N	Zscore	P-value
family.Christensenellaceae.id.1866	-0.59	0.12	4.56E-07	8.04E-05	-0.10	0.06	1.32E-01	2593	-1.88	5.96E-02
genus.ChristensenellaceaeR7group.id.11283	-0.55	0.11	3.31E-07	8.04E-05	-0.10	0.06	1.30E-01	2593	-4.42	9.80E-06
genus.RuminococcaceaeUCG005.id.11363	**-0.59**	**0.13**	**1.36E-05**	**1.57E-03**	**-0.12**	**0.07**	**7.62E-02**	**2593**	**-4.14**	**3.48E-05**
genus.RuminococcaceaeUCG010.id.11367	-0.50	0.12	1.77E-05	1.57E-03	-0.07	0.06	2.08E-01	2593	-3.71	2.11E-04
family.Ruminococcaceae.id.2050	**-1.59**	**0.38**	**2.51E-05**	**1.77E-03**	**-0.52**	**0.24**	**3.11E-02**	**2593**	**-4.35**	**1.38E-05**
genus.Coprococcus2.id.11302	-0.29	0.08	2.74E-04	1.03E-02	-0.02	0.04	5.79E-01	2593	-2.75	6.02E-03
genus.FamilyXIIIAD3011group.id.11293	-0.74	0.20	2.56E-04	1.03E-02	-0.06	0.10	5.78E-01	2593	-2.76	5.78E-03
genus.Lachnoclostridium.id.11308	**0.57**	**0.16**	**3.11E-04**	**1.03E-02**	**0.27**	**0.11**	**1.80E-02**	**2593**	**4.12**	**3.76E-05**
genus.RuminococcaceaeUCG002.id.11360	**-0.42**	**0.12**	**3.21E-04**	**1.03E-02**	**-0.16**	**0.07**	**2.33E-02**	**2593**	**-4.04**	**5.30E-05**
genus.RuminococcaceaeUCG003.id.11361	**-0.51**	**0.15**	**4.60E-04**	**1.33E-02**	**-0.25**	**0.08**	**3.10E-03**	**2593**	**-4.51**	**6.42E-06**
class.Mollicutes.id.3920	-0.39	0.12	8.63E-04	1.38E-02	-0.01	0.05	8.04E-01	2593	-2.32	2.06E-02
genus.Eggerthella.id.819	**0.65**	**0.19**	**6.28E-04**	**1.38E-02**	**0.31**	**0.12**	**1.18E-02**	**2593**	**4.12**	**3.80E-05**
genus.Hungatella.id.11306	**0.77**	**0.22**	**6.73E-04**	**1.38E-02**	**0.34**	**0.14**	**1.34E-02**	**2593**	**4.07**	**4.65E-05**
genus.Ruminiclostridium6.id.11356	-0.36	0.11	6.23E-04	1.38E-02	-0.01	0.05	9.06E-01	2593	-2.27	2.31E-02
genus.RuminococcaceaeUCG014.id.11371	-0.29	0.08	5.70E-04	1.38E-02	-0.05	0.05	3.16E-01	2593	-2.97	2.99E-03
order.MollicutesRF9.id.11579	-0.40	0.12	8.28E-04	1.38E-02	0.00	0.05	9.55E-01	2593	-2.18	2.96E-02
phylum.Tenericutes.id.3919	-0.39	0.12	8.63E-04	1.38E-02	-0.01	0.05	8.04E-01	2593	-2.32	2.06E-02
genus.Coprococcus3.id.11303	**-0.43**	**0.13**	**9.09E-04**	**1.39E-02**	**-0.29**	**0.09**	**1.20E-03**	**2593**	**-4.61**	**4.04E-06**
genus.LachnospiraceaeUCG001.id.11321	**-0.52**	**0.16**	**1.01E-03**	**1.48E-02**	**-0.16**	**0.07**	**2.34E-02**	**2593**	**-3.84**	**1.21E-04**
family.Micrococcaceae.id.636	1.53	0.47	1.26E-03	1.71E-02	-0.14	0.16	3.75E-01	2593	2.92	3.55E-03
genus..Ruminococcusgauvreauiigroup.id.11342	**-0.34**	**0.11**	**1.54E-03**	**2.01E-02**	**-0.13**	**0.07**	**4.90E-02**	**2593**	**-3.54**	**4.06E-04**
genus..Eubacteriumventriosumgroup.id.11341	**-0.49**	**0.16**	**2.09E-03**	**2.47E-02**	**-0.17**	**0.08**	**4.20E-02**	**2593**	**-3.53**	**4.19E-04**

Significantly associated taxa with depressive symptom levels in both Rotterdam Study (N = 1,054) and HELIUS (N = 1,539) cohorts. Linear regression models were used adjusting for several life-style confounders (such as smoking and alcohol use) and medical confounders (such as PPI, metformin).

	Rotterdam Study (N = 1054)				HELIUS (N = 1539)			Meta-analysis		
genus.Sellimonas.id.14369	**0.57**	**0.18**	**2.10E-03**	**2.47E-02**	**0.54**	**0.13**	**3.55E-05**	**2593**	**5.15**	**2.65E-07**
genus.Rothia.id.646	1.47	0.48	2.21E-03	2.52E-02	0.32	0.23	1.66E-01	2593	3.02	2.55E-03
order.Micrococcales.id.510	1.41	0.47	2.58E-03	2.84E-02	-0.21	0.15	1.68E-01	2593	2.96	3.09E-03
genus.LachnospiraceaeNK4A136group.id.11319	-0.41	0.14	2.80E-03	2.99E-02	-0.08	0.08	3.48E-01	2593	-2.63	8.56E-03
genus.Subdoligranulum.id.2070	**-0.44**	**0.15**	**3.36E-03**	**3.48E-02**	**-0.28**	**0.09**	**1.66E-03**	**2593**	**-4.29**	**1.77E-05**
genus.RuminococcaceaeNK4A214group.id.11358	-0.34	0.12	4.66E-03	4.70E-02	-0.04	0.06	5.73E-01	2593	-2.24	2.52E-02
genus.PrevotellaceaeUCG001.id.11186	-0.49	0.17	4.80E-03	4.71E-02	-0.02	0.08	8.38E-01	2593	-1.96	5.06E-02

Significantly associated taxa with depressive symptom levels in both Rotterdam Study (N = 1,054) and HELIUS (N = 1,539) cohorts. Linear regression models were used adjusting for several life-style confounders (such as smoking and alcohol use) and medical confounders (such as PPI, metformin).

Random forest analysis with RS as the training cohort and HELIUS as the testing cohort revealed *RuminococcaceaeUCG005* as the most important genus in predicting depressive symptoms (**Supplementary Table 4**), showing the highest percentage increase in mean squared error (%incMSE) in out of bag analysis. Other important predictors of depressive symptoms include *ChristensenellaceaeR7group, Lachnoclostridium, Eggerthella, Sellimonas*, and *Hungatella*, which overlap with the findings of the linear regression analysis in this study (**Supplementary Table 4**). Further, important predictors identified by random forest analysis include *Roseburia*, *Streptococcus*, *Bacteroides*, *Anaerotruncus*, *Dorea*, *Blautia*, *Veillonella*, *Desulfovibrio*, *Anaerostipes* and *Bifidobacterium*, which replicate associations reported earlier [39].

Mendelian Randomization (MR) analysis

Results of MR analysis with major depression and microbiome as exposures are provided in the Supplementary Tables 5 & 6 respectively. With major depression as the exposure, *Eggerthella, Sellimonas* and *Hungatella* showed nominally significant MR results (**Supplementary Table 5**). For *Eggerthella*, both simple median and IVW method showed nominally significant MR results with consistent effect estimates. *Sellimonas* showed nominally significant result under the simple median method. Further the effect estimates for *Eggerthella* and *Sellimonas* were also consistent with the findings of this study, i.e., increase in the abundance of these two genera in those with higher depressive symptoms. For *Hungatella*, only MR-Egger method showed significant results and a significant intercept suggesting pleiotropy/heterogeneity. With microbiome as exposure, significant MR was observed for genus *Sellimonas* under the median and IVW methods but effect estimates were inconsistent with the findings of our study (**Supplementary Table 6**).

Among the 87 depression-associated SNPs [7] significant association was observed for one SNP rs17641524 with the genus *Acidaminococcus* after multiple testing correction (**Supplementary Table 7**). No significant association was observed for the MDD GRS (**Supplementary Table 8**).

Discussion

In this large study of 2593 individuals profiled for depressive symptoms and microbiome, we identified 12 genera and 1 microbial family associated with depression. These include genus *Sellimonas, Eggerthella, Ruminococcaceae (UCG002, UCG003, UCG005), Lachnoclostridium, Hungatella, Coprococcus, LachnospiraceaeUCG001, Ruminococcusgauvreauiigroup, Eubacterium ventriosum, Subdoligranulum and family Ruminococcaceae. Sellimonas, Eggerthella, Lachnoclostridium and Hungatella* were more abundant in individuals with more severe depressive symptoms. All other taxa were depleted in depression. Alpha diversity was significantly associated with depressive symptoms in both discovery and replication cohorts.

The intestinal bacterial strains *Eggerthella, Subdoligranulum, Coprococcus* and *Ruminococcaceae* have been reported to be associated with depression in earlier studies. *Eggerthella* has been reported to be consistently found to be increased in depression and anxiety cases in 8 studies [26, 27, 39], which is in line with the findings of our study. MR analysis suggests that increase abundance of *Eggerthella* in depression is more likely to be a consequence of the disease rather than a cause. Also in line with our findings *Subdoligranulum* and *Coprococcus* were consistently found to be depleted in individuals with generalized anxiety disorder and depression several studies [39]. Both *Subdoligranulum*

and *Coprococcus* are involved in the production of butyrate [27] and *Subdoligranulum* was found to be increased in omega 3 rich diet [40]. *Ruminococcaceae* at genus and family levels have been found to be depleted in cases of both uni- and bipolar depression [26, 27, 39, 41–43].

Novel findings of this study include genera *Sellimonas*, *Lachnoclostridium*, *Hungatella*, *Eubacterium* v*entriosum*, *Subdoligranulum*, *LachnospiraceaeUCG001*, and *Ruminococcusgauvreauiigroup*. *Sellimonas* and *Hungatella* were positively associated with depressive symptoms. *Sellimonas* is the most significant finding of this study. It belongs to the family Lachnospiraceae and phylum Firmicutes. Species belonging to *Sellimonas* have been found to be increased in inflammatory diseases including ankylosing spondylitis, atherosclerosis and liver cirrhosis [44]. Further, increased abundance of *Sellimonas* have been observed after dysbiosis [45]. *Lachnoclostridium*, which also belongs to the family Lachnospiraceae, appeared as the topmost important predictor of depressive symptoms in random forest analysis. Higher levels of *Lachnoclostridium* were associated with increased depressive symptoms in our study. *Lachnoclostridium* has previously found to be depleted in other psychiatric disorders including schizophrenia [46] and autism [47] and in patients with gastrointestinal tract neoplasms [48]. *Hungatella* was found to be associated with paleolithic diet and is known to produce the precursor molecule for trimethylamine-N-oxide (TMAO) [49]. TMAO has been implicated in cardio-vascular and neurological diseases including depression [50, 51]. *Eubacterium ventriosum* belongs to the family Eubacteriaceae and has been found to be significantly depleted after traumatic brain injury in mice [52]. Major depression is a frequent complication of traumatic brain injury [53]. In our study we also observed depletion of *Eubacterium ventriosum* with the increase in depressive symptoms, which fits well with association with traumatic brain injury. In human studies *Eubacterium ventriosum* was found to be slightly more abundant in obese individuals [54, 55]. Obesity is one of the most prevalent somatic comorbidities of major depressive disorder [56, 57] and is partly attributed to a side effect of selective serotonin reuptake inhibitors (SSRI). However, in our study we excluded those using antidepressant and adjusted for BMI in the linear regression analysis thus our finding is independent of the association with body weight. *Subdoligranulum* belongs to the family Ruminococcaceae. *LachnospiraceaeUCG001*, at species level, was found to be associated with anhedonia in mice [58]. *Ruminococcusgauvreauii* belongs to the family Ruminococcaceae and at species level was found to be increased in atherosclerotic conditions [44].

Most identified microbiota in our study show potential involvement in the synthesis of glutamate and butyrate (see Supplementary Table 12 of Valles-Colomer et al. 2019) [27]. *Eggerthella* is further involved in the synthesis of serotonin and gamma aminobutyric acid (GABA). Glutamate is widely distributed in the brain and a major excitatory synaptic neurotransmitter[59]. It is known to be involved in regulating neuroplasticity, learning and memory [60]. Glutamate levels in plasma, serum, cerebrospinal fluid and brain tissue have been associated with mood and psychotic disorders and suicide [61–66]. With increasing evidence of its role in the etiology of depressive disorders, glutamate is rapidly becoming the novel therapeutic target for depressive disorders. Ketamine, for instance, has been shown to increase glutamate signaling in rodents and humans [67, 68] and has shown to reduce depressive symptoms rapidly [69]. Glutamate plays a role as a neurotransmitter in the enteric nervous system, which sustains the reciprocal influence between the gastrointestinal tract and the central nervous system [8, 70]. Butyrate on the hand is a short chain fatty acid and modulates biological responses of host gastrointestinal health by acting as a histone deacetylase inhibitor and binding to specific G protein-coupled receptors (GPCRs) [71]. Butyrate can affect the gut-brain axis by enhancing the cholinergic neurons via epigenetic mechanisms [72] and can cross the blood brain barrier and activate the vagus nerve and hypothalamus [73, 74]. Sodium butyrate has shown anti-depressant effects in animal models of depression and mania [75, 76]. Serotonin and GABA are both important neurotransmitters relevant to depression. Evidence suggests that serotonin may be the key neurotransmitter to the gut-brain axis [77]. Enteric nervous system accounts for > 90% of the body's serotonin production where it is produced by enterochromaffin cells and in the neurons of the enteric nervous system [78]. The neuronal production of serotonin is most critical for the development and motility of the enteric nervous system, affecting neurogenesis and guiding development of neurons expressing dopamine and GABA [78–80]. Although serotonin produced by the gut cannot cross the blood-brain barrier [81], it can affect the blood-brain barrier permeability, which can lead to inflammation of the brain [82]. Further, vagus nerve stimulation by the gut microbiota can alter concentration of serotonin, GABA and glutamate within the brain in animals and humans [51, 83] and germ-free male mice exhibit anxiety-like behaviors and altered serotonin abundance in the brain [15]. GABA is the main inhibitory neurotransmitter of the central nervous system that counterbalances the action of glutamate [84]. Low levels of GABA are linked to depression and mood disorders [84]. Animal studies show that gut microbiota can alter GABA activity in the brain through the vagus nerve [85]. While each of the metabolites mentioned above are highly relevant for depression, most are known to be unable to cross the blood-brain barrier. However, an increasing number of animal studies show that the peripheral production of neurotransmitters by the gut microbiome can alter brain chemistry and therefore influence mood and behavior [51].

In the current study, we aimed to identify gut microbiota associated with depression in the general population to overcome the bias of reversed causation. The strengths of our study include a large sample, controlling for most known confounders including comorbid conditions, performing analysis in individuals free of anti-depressive medication and finally the use of quantitative depression scales. A large study consisting of 252,503 individuals from 68 countries showed that subthreshold depressive disorders produce significant decrements in health and do not qualitatively differ from full-blown episodes of depression [86]. Use of rating scales is thus more powerful in omics association studies [87]. There may have been a loss of statistical power as the depression assessment scales were different in the discovery and replication cohorts. Further, despite the use of the largest GWAS for both microbiome and depression, the MR analysis lacked power. There are

87 SNPs identified for depression, however, their effect on depression is small (individual odds ratio < 1.05, combined odds ratio < 2.0), which makes unlikely that the individual genetic variants show association with microbiome. For microbiome, there were no SNPs significantly associated at the genome-wide level and we had to lower the threshold to 10^{-05} to identify at least more than one independent instrument for the identified microbiota. This limits the value of the MR.

To summarize, we have identified several bacteria at genera level that might influence depression in humans. We confirm the association of *Eggerthella, Coprococcus*, *Subdoligranulum* and family Ruminococcaceae and identify novel bacteria including *Sellimonas, Lachnoclostridium, Hungatella, Ruminococcus, Subdoligranulum, LachnospiraceaeUCG001, Eubacterium ventriosum* and *Ruminococcusgauvreauiigroup*. These bacteria are involved in the synthesis of glutamate, butyrate, serotonin and GABA, which are the key neurotransmitters relevant for depression.

Declarations

Acknowledgements

The Rotterdam Study is a population-based cohort study from the well-defined Ommoord district within Rotterdam, The Netherlands. It is designed to investigate occurrence and determinants of diseases in the elderly. The RS cohort is approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study was entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. We thank all participants and all others, who made this study possible.

The HELIUS study is conducted by the Amsterdam University Medical Centers, location AMC and the Public Health Service of Amsterdam. Both organisations provided core support for HELIUS. The HELIUS study is also funded by the Dutch Heart Foundation, the Netherlands Organization for Health Research and Development (ZonMw), the European Union (FP-7), and the European Fund for the Integration of non-EU immigrants (EIF). We are most grateful to the participants of the HELIUS study and the management team, research nurses, interviewers, research assistants and other staff who have taken part in gathering the data of this study. Grant numbers:

- Dutch Heart Foundation: 2010T084 (K Stronks)

- ZonMw: 200500003 (K Stronks)

- European Union (FP-7): 278901 (K Stronks)

- European Fund for the Integration of non-EU immigrants (EIF): 2013EIF013 (K Stronks)

Conflict of interest

The authors declare no conflict of interest

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