The World Journal of Biological Psychiatry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iwbp20

Sex specific effect of gut microbiota on the risk of psychiatric disorders: A Mendelian randomisation study and PRS analysis using UK Biobank cohort

Xin Qi, Fanglin Guan, Shiqiang Cheng, Yan Wen, Li Liu, Mei Ma, Bolun Cheng, Chujun Liang, Lu Zhang, Xiao Liang, Ping Li, Xiaomeng Chu, Jing Ye, Yao Yao & Feng Zhang

To cite this article: Xin Qi, Fanglin Guan, Shiqiang Cheng, Yan Wen, Li Liu, Mei Ma, Bolun Cheng, Chujun Liang, Lu Zhang, Xiao Liang, Ping Li, Xiaomeng Chu, Jing Ye, Yao Yao & Feng Zhang (2021) Sex specific effect of gut microbiota on the risk of psychiatric disorders: A Mendelian randomisation study and PRS analysis using UK Biobank cohort, The World Journal of Biological Psychiatry, 22:7, 495-504, DOI: 10.1080/15622975.2021.1878428

To link to this article: https://doi.org/10.1080/15622975.2021.1878428

THE WORLD JOURNAL OF BIOLOGICAL PSYCHIATRY
2021, VOL. 22, NO. 7, 495–504
https://doi.org/10.1080/15622975.2021.1878428

REVIEW ARTICLE

Sex specific effect of gut microbiota on the risk of psychiatric disorders: A Mendelian randomisation study and PRS analysis using UK Biobank cohort

Xin Qi[a,b]*, Fanglin Guan[c]*, Shiqiang Cheng[a], Yan Wen[a], Li Liu[a], Mei Ma[a], Bolun Cheng[a], Chujun Liang[a], Lu Zhang[a], Xiao Liang[d], Ping Li[a], Xiaomeng Chu[a], Jing Ye[a], Yao Yao[a] and Feng Zhang[a]

[a]Key Laboratory of Trace Elements and Endemic Diseases of National Health and Family Planning Commission, School of Public Health, Health Science Center, Xi'an Jiaotong University, Xi'an, P. R. China; [b]Precision Medicine Center, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, P. R. China; [c]School of Medicine and Forensics, Xi'an Jiaotong University, Xi'an, P. R. China; [d]The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, P. R. China

ABSTRACT

Objective: The relationships between gut microbiota and brain-related diseases/traits remains not fully understood.

Method: A two-stage study was performed to investigate the relationships between gut microbiota and brain-related diseases/traits, and evaluate the potential sex specific effects of gut microbiota. In discovery stage, we systematically scanned the relationships between 515 brain-related diseases/traits and gut microbiota through two-sample Mendelian randomisation analysis. Using ~500,000 individuals derived from the UK Biobank, polygenetic risk scoring (PRS) analysis was performed to validate the associations detected in discovery stage. To evaluate the potential sex-specific effect of gut microbiota on brain-related disorders, PRS analysis was conducted in female and male, respectively.

Results: After systematically scanning diseases or traits, 41 of the 515 brain-related diseases/traits were identified to be associated with gut microbiota, such as Neuroticism score ($P_{2\text{-}MR} = 0.0018$), worrier/anxious feelings ($P_{2\text{-}MR} = 0.0013$), Suffer from 'nerves' ($P_{2\text{-}MR} = 0.0062$) and Nervous feelings ($P_{2\text{-}MR} = 0.0158$). 5 of 41 brain-related diseases or traits were successfully validated in UK Biobank, such as Neuroticism score ($P_{UK} = 0.0024$, $P_{UK\text{-}female} = 0.0063$, $P_{UK\text{-}male} = 0.1142$), Nervous feelings ($P_{UK} = 0.0043$, $P_{UK\text{-}female} = 0.0115$, $P_{UK\text{-}male} = 0.1670$) and Worrier/anxious feelings ($P_{UK} = 0.0166$, $P_{UK\text{-}female} = 0.0196$, $P_{UK\text{-}male} = 0.2930$).

Conclusion: Our results suggest that gut microbiota contributed more to brain-related diseases or traits in females than in males.

KEY POINTS

- A two-stage study was performed to investigate the relationships between gut microbiota and brain-related diseases/traits.
- Using the individuals derived from the UK Biobank, polygenetic risk scoring analysis was performed to validate the associations detected in the discovery stage.
- Our results suggest that gut microbiota contributed more to brain-related diseases or traits in females than in males.

ARTICLE HISTORY
Received 24 August 2020
Accepted 15 December 2020

KEYWORDS
Gut microbiota; brain; sex specific; psychiatric disorders; neurological disorders

Introduction

Brain is recognised as the centre of nervous system in humans. Brain function is controlled and realised through interconnecting neurons arranged in cerebral cortex and deep brain nuclei (Larvie and Fischl 2016). Psychiatric disorders are a group of syndromes affecting mood, thinking and behaviour of individuals, such as bipolar disorders, schizophrenia, autism spectrum disorders and major depressive disorders (MDD). Neurological disorders are the dysfunction of nerve system, such as Alzheimer's disease, epilepsy, and Parkinson's disease. The previous literatures suggested that genetic factors have a vital role in aetiology of psychiatric disorders and neurological disorders (Cross-Disorder Group of the Psychiatric Genomics et al. 2013; Han et al. 2014). 29.2% and 6% of population

CONTACT Feng Zhang fzhxjtu@mail.xjtu.edu.cn School of Public Health, Health Science Center, Xi'an Jiaotong University, No. 76 Yan Ta West Road, Xi'an, 710061, P. R. China
*The two authors contributed equally to this work.
Supplemental data for this article can be accessed at https://doi.org/10.1080/15622975.2021.1878428

were discovered to show psychiatric disorder and neurological disorder, respectively, which were higher than other common diseases (MacDonald et al. 2000; Steel et al. 2014). Neurological disorders displayed a component of 6.3% to global burden of disease, and psychiatric and neurological disorders cause a substantial and heavy financial and medical burden to patients and caregivers (OWH 2010; Sagar et al. 2020).

The genetic and environmental factors all contributed to the aetiology of psychiatric and neurological disorders. The SNP heritability (h○2[SNP]) between psychiatric and neurological disorders estimated by genetic correlation analysis was around 0.2, and there are cases for bipolar disorder and schizophrenia, with heritability of around 85% and 80%, respectively (McGuffin et al. 2003; Sullivan et al. 2003; Cross-Disorder Group of the Psychiatric Genomics et al. 2013; Anttila et al. 2018; Hilker et al. 2018). For psychiatric and neurological disorders, genome-wide association studies (GWAS) have focussed on its genetic basis, and found that genetic factors have significant efforts on aetiology of psychiatric and neurological disorders (Blauwendraat et al. 2019; Horwitz et al. 2019). Two new risk loci (*ADCY2*, and a region between *MIR2113* and *POU3F2*) were identified to be susceptible for bipolar disorder through GWAS analysis (Mühleisen et al. 2014). Moreover, the risk factors in environment have a vital impact on the development of psychiatric and neurological disorders, such as alcohol dependence, smoke and risk taking (Heffner et al. 2011; Schmitt et al. 2014; Walters et al. 2018). The previous GWAS literature discovered that there were significant genetic correlations between alcohol dependence and psychiatric disorders, including schizophrenia, attention deficit–hyperactivity disorder, and depression (Walters et al. 2018). Jaimee et al. found that compared with individuals without psychiatric disorders, bipolar disorder patients were 2–3 times more likely to have nicotine dependence and were less likely to have smoking cessation (Heffner et al. 2011).

Gut is the biggest residence of human microbiota, and it has a unique combination of species for every individual (Ursell et al. 2012). The regulation of genetic factors has an influence on the development of gut microbiota, and gut microbiota were found to be associated with psychiatric disorders, such as depression and autistic spectrum disorder (Cong et al. 2016; Clapp et al. 2017). Researches have shown that gut microbiota have profound effects on the brain and behaviour via gut-brain-axis: through neuronal activation, endocrine signals, and immune pathways (Clapp et al. 2017; Valles-Colomer et al. 2019; Wang et al. 2019). Two bacteria genus, named *Coprococcus* and *Dialister*, were found to be depleted in gut microbiota of depression people (Valles-Colomer et al. 2019). Moreover, a randomised, double-blinded and placebo-controlled trial literature researched that only *Bifidobacterium longum* 1714^{TM} could alter neural activity in brain areas, which indicated that *B. longum* 1714^{TM} modulated resting neural activity and reduced mental fatigue (Wang et al. 2019).

Mendelian randomisation (MR) is a method that aims to assess relationships between risk factors and diseases using genetic variants as instrumental variables. Two-sample MR can obtain the effect of the instrumental variable-risk factor association and instrumental variable-outcome association from different participants. In two-sample MR, using GWAS summary data can increase statistical power, and especially test effects on binary disease outcomes. Using two-sample MR, Choi et al. (2020) found that there existed significant causal relationship between high levels of bilirubin and decreased stroke risk.

In this study, we carried out a two-stage analysis to assess whether there existed sex differences in relationships between gut microbiota and brain-related diseases or traits. First, we systematically scanned 515 brain-related diseases or traits, and investigated their relationships with gut microbiota through two-sample Mendelian randomisation (MR) analysis. Second, using ~500,000 individuals from UK Biobank, polygenetic risk scoring (PRS) analysis was performed to validate the significant associations of discovery stage for female and male, respectively.

Materials and methods

UK biobank dataset

Ethics approval

The UK Biobank study was approved by the National Health Service National Research Ethics Service (11/NW/0382). To participate in the UK Biobank study, all participants have supplied informed consent. Our data dictionary ID for application is 46478.

Study population, genotype, imputation and quality control

A cohort study of ~500,000 individuals from the UK composed of UK Biobank source, which were all aged 40 to 69 years. From over 9.2 million invitations, 503,325 participants were recruited. Participants used touchscreen tests and questionnaires as well as nurse-led interviews to self-report the extensive phenotypic

data upon baseline assessment. UK BiLEVE Axiom Array or the UK Biobank Axiom Array was used to genotype (The UK Biobank Array Design Group 2014; Wain et al. 2015). PLINK v2.0 and R v3.5.1 were used to perform quality control (Chang et al. 2015). All subjects included in this study are self-reported white people. The samples were excluded because of sex chromosome karyotypes putatively different from XY or XX. Therefore, 450,580 samples (244,945 females and 205,635 males) passed sample quality control. With the 1000 Genomes Phase 3 dataset as the reference panel, SHAPEIT3 was used to perform imputation (McVean et al. 2012; O'Connell et al. 2016). Imputation files were released in the BGEN (v1.2) file format (Band and Marchini 2018). For the UK Biobank study, the detailed description of the genotyping, quality control and imputation are described in previous study (McVean et al. 2012; The UK Biobank Array Design Group 2014; Chang et al. 2015; Wain et al. 2015; O'Connell et al. 2016; Band and Marchini 2018; Bycroft et al. 2018).

Outcome definition

Seven self-reported outcomes from UK Biobank were used in our study: Neuroticism score, Fluid intelligence score, Suffer from 'nerves', Alcohol intake frequency (never, special occasions only, 1–3 times/month, 1–2 times/week, 3–4 times/week, daily), Worrier/anxious feelings, Nervous feelings and Worry too long after embarrassment. The detailed number of samples for these outcomes was listed in Supplemental Table 1.

GWAS datasets of gut microbiota

Two recent large-scale GWAS summary datasets of gut microbiota were used in this study (Goodrich et al. 2016; Turpin et al. 2016). Detailed description of cohorts, genotyping, imputation, and quality control approaches could be found in the previous studies. In brief, 2,731 subjects and 1,561 subjects were included in two GWAS datasets respectively. The Illumina Miseq platform with 2×250bp paired-end sequencing was used to sequence the pooled amplicons. QIIME version 1.8 was used to perform mate-pair merging, de-multiplexing, quality control and operational taxonomic units (OUT) picking (Caporaso et al. 2010). IMPUTE version 2 or HumanCoreExome-12v1.1 chip (Illumina) was used to genotype and impute for individuals (Howie et al. 2009). SNP-microbe association tests were performed using GEMMA (v0.94) (Zhou and Stephens 2012). In total, we selected 306 significant SNPs with $p < 5.0 \times 10^{-5}$ from two GWAS results (Supplementary Table 2).

Discovery study: two-sample Mendelian randomisation (MR) analysis

In discovery stage, we performed a two-sample MR analysis to explore the relationships between gut microbiota and brain-related diseases or traits (Supplementary Table 3) (Goodrich et al. 2016; Turpin et al. 2016; Hemani et al. 2018). GWAS datasets of 515 brain-related diseases or traits were derived from MR-Base (http://www.mrbase.org), which composed 11 billion SNP-trait associations from 1673 GWAS (Hemani et al. 2018). We selected 306 gut microbiota related SNPs as exposure (Supplementary Table 2). Five hundred and fifteen brain-related diseases or traits were selected as outcome (Supplementary Table 3). Based on these datasets, the causal effects of exposure on the outcome could be estimated. Finally, we performed two-sample MR analysis and Wald ratios were calculated for each SNP in WebApp.

Some previous researches have used MR analysis in microbiome field. For example, Iraia et al. used two-sample Mendelian Randomisation analysis to investigate associations between gut microbiota and coeliac disease in the published study (García-Santisteban et al. 2020). Besides, Senara et al. performed MR analysis and found that the host-genetic-driven increase in gut production of the short-chain fatty acid butyrate was associated with improved insulin response after an oral glucose-tolerance test (Sanna et al. 2019). Three different MR methodologies with varying assumptions of instrument validity were used in this study: inverse variance weighted approach (IVW) as main analysis method, and MR-Egger and weighted median estimation as sensitivity analysis. (1) IVW MR method, which effectively treats each SNP as a valid natural experiment. An unbiased causal estimate could be obtained by IVW MR, if there is no horizontal pleiotropy, or horizontal pleiotropy is balanced. The contribution of each instrumental SNP to overall effect is weighted by the inverse of variance of SNP-outcome effect. (2) MR-Egger regression allows the intercept to pass through a value other than zero to relax this constraint, when there has a tendency for unbalanced horizontal pleiotropy. (3) The weighted median approach was also applied. The median based approach provides an unbiased estimate in presence of unbalanced horizontal pleiotropy, when the majority of instruments are valid and some are invalid instruments. We used clumping to prune SNPs for

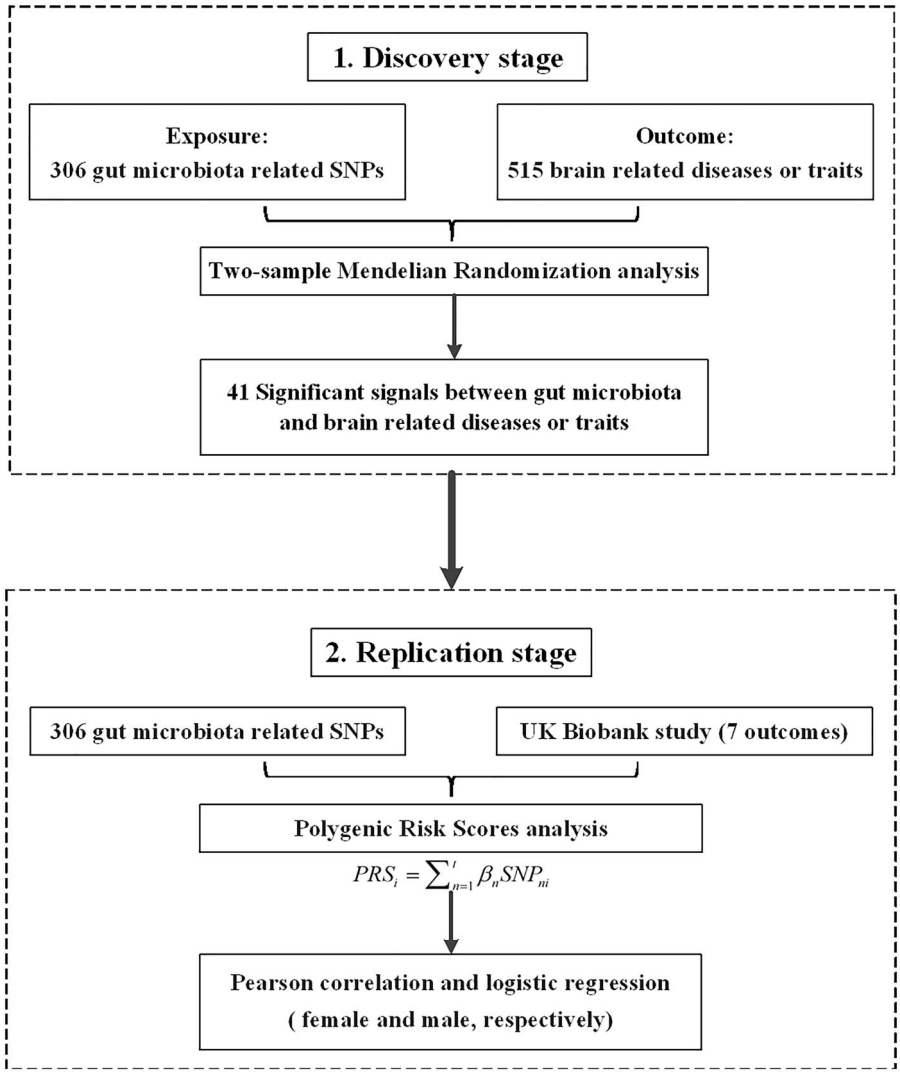

Figure 1. The flowchart of this study.

Linkage disequilibrium (LD). Minimum LD Rsq value is $p > 0.8$. Palindromic SNPs were allowed with Minor Allele Frequency (MAF) threshold >0.3. $p < 0.05$ was selected as the significant level for three methodologies, and $p > 0.05$ was significant for horizontal pleiotropy (HP). The flowchart of this study is shown in Figure 1.

Replication study: polygenic risk scores (PRS) analysis

To further confirm the significant results of discovery stage, UK Biobank dataset was used to perform PRS analysis. PRS is an algorithm which adds up the effect of risk alleles associated with a outcome in each individual (Liu et al. 2018). Similar study design has been used in the previous studies of complex diseases (Mavaddat et al. 2019; Kujala et al. 2020). In this approach, we calculated each individual's PRS of gut microbiota using the UK Biobank dataset. We defined PRS_i as the PRS value of gut microbiota for the i ($i = 1,2,3,\ldots,k$) individual, defined as $PRS_i = \sum_{n=1}^{t} \beta_n SNP_{ni}$. β_n represents effect value of risk allele of n*th* ($n = 1,2,3,\ldots,t$) significant SNP associated with gut microbiota, which was obtained from previous research (Goodrich et al. 2016; Turpin et al. 2016). SNP_{ni} denotes risk allele dosage of n*th* SNP of i*th* individual. Then, PRS of gut microbiota was obtained for each individual in the UK Biobank study. PRS analysis was performed in PLINK 2.0 (Chang et al. 2015). We verified the outcomes (Please see above *outcome definition*) from the significant discovery results. Pearson correlation and logistic regression analysis were conducted to evaluate the associations between gut microbiota and 7 outcomes identified in discovery study by using calculated PRS as instrumental

Table 1. The significant brain-related traits or diseases identified by two-sample MR analysis.

		Horizontal pleiotropy			Inverse variance weighted		
Id.	Outcome	Egger regression intercept:	SE	*p* Value	B	SE	*p* Value
1065	Inspection time	9.50×10^{-4}	2.90×10^{-3}	0.7440	-1.10×10^{-3}	3.12×10^{-4}	0.0004
UKB-a:53	Worry too long after embarrassment	2.00×10^{-5}	1.50×10^{-4}	0.8950	-5.91×10^{-5}	1.68×10^{-5}	0.0004
UKB-b:13653	Worry too long after embarrassment	-5.60×10^{-7}	1.30×10^{-4}	0.9970	-4.48×10^{-5}	1.46×10^{-5}	0.0021
UKB-b:6787	Diagnoses - secondary ICD10: K70.4 Alcoholic hepatic failure	-3.10×10^{-6}	1.70×10^{-6}	0.0785	-5.78×10^{-7}	1.89×10^{-7}	0.0023
UKB-b:10407	Diagnoses - main ICD10: K70.0 Alcoholic fatty liver	2.50×10^{-6}	1.40×10^{-6}	0.0830	4.34×10^{-7}	1.57×10^{-7}	0.0056
UKB-a:51	Worrier / anxious feelings	-5.90×10^{-5}	1.40×10^{-4}	0.6720	-4.86×10^{-5}	1.51×10^{-5}	0.0013
UKB-b:15294	Diagnoses - secondary ICD10: F43.1 Posttraumatic stress disorder	1.90×10^{-6}	2.40×10^{-6}	0.4410	-6.76×10^{-7}	2.62×10^{-7}	0.0098
UKB-b:14993	Diagnoses - main ICD10: F23.0 Acute polymorphic psychotic disorder without symptoms of schizophrenia	-3.60×10^{-7}	1.30×10^{-6}	0.7840	-4.09×10^{-7}	1.43×10^{-7}	0.0042
UKB-b:4908	Diagnoses - secondary ICD10: G40.1 Localisation-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures	1.20×10^{-6}	1.90×10^{-6}	0.5260	5.58×10^{-7}	2.11×10^{-7}	0.0083
UKB-b:18910	Diagnoses - secondary ICD10: G99.0 Autonomic neuropathy in endocrine and metabolic diseases	-4.70×10^{-6}	2.70×10^{-6}	0.0839	-7.72×10^{-7}	2.94×10^{-7}	0.0086
UKB-b:2253	Diagnoses - secondary ICD10: T43.0 Tricyclic and tetracyclic antidepressants	1.50×10^{-6}	3.50×10^{-6}	0.6700	-1.02×10^{-6}	3.86×10^{-7}	0.0082
UKB-b:7921	Diagnoses - secondary ICD10: K70.3 Alcoholic cirrhosis of liver	-3.50×10^{-6}	4.60×10^{-6}	0.4500	-1.27×10^{-6}	4.99×10^{-7}	0.0111
UKB-b:6519	Worrier / anxious feelings	-1.30×10^{-5}	1.30×10^{-4}	0.9180	-3.99×10^{-5}	1.36×10^{-5}	0.0035
UKB-a:230	Neuroticism score	8.20×10^{-4}	1.20×10^{-3}	0.4840	-3.94×10^{-4}	1.26×10^{-4}	0.0018
UKB-a:20	Exposure to tobacco smoke outside home	2.80×10^{-4}	1.80×10^{-4}	0.1200	-4.84×10^{-5}	1.97×10^{-5}	0.0138
UKB-b:16343	Treatment speciality of consultant (recoded): Forensic psychiatry	8.30×10^{-7}	1.40×10^{-6}	0.5420	3.25×10^{-7}	1.48×10^{-7}	0.0275
UKB-a:18	Smoking/smokers in household	6.20×10^{-5}	9.30×10^{-5}	0.5060	-2.52×10^{-5}	1.01×10^{-5}	0.0126
UKB-a:50	Nervous feelings	-1.30×10^{-5}	1.30×10^{-4}	0.9190	-3.30×10^{-5}	1.37×10^{-5}	0.0158
UKB-a:200	Suffer from 'nerves'	2.60×10^{-5}	1.20×10^{-4}	0.8320	-3.63×10^{-5}	1.33×10^{-5}	0.0062
UKB-b:4002	Treatment/medication code: polyvinyl alcohol 1% eye drops	3.70×10^{-6}	2.80×10^{-6}	0.1860	-6.39×10^{-7}	3.05×10^{-7}	0.0361
UKB-b:5238	Fluid intelligence score	-1.30×10^{-3}	1.00×10^{-3}	0.2100	2.49×10^{-4}	1.12×10^{-4}	0.0269
UKB-a:330	Number of unsuccessful stop-smoking attempts	8.10×10^{-4}	4.70×10^{-4}	0.0855	-1.06×10^{-4}	5.10×10^{-5}	0.0380
UKB-b:1823	Diagnoses - main ICD10: F32.2 Severe depressive episode without psychotic symptoms	1.10×10^{-6}	4.30×10^{-6}	0.7920	-1.02×10^{-6}	4.66×10^{-7}	0.0280
UKB-b:10630	Operative procedures - secondary OPCS: A68.4 Primary neurolysis of peripheral nerve NEC	2.70×10^{-6}	1.70×10^{-6}	0.1130	-4.23×10^{-7}	1.86×10^{-7}	0.0227
UKB-b:4630	Neuroticism score	5.10×10^{-4}	1.00×10^{-3}	0.6200	-3.17×10^{-4}	1.13×10^{-4}	0.0049
UKB-a:25	Alcohol intake frequency.	1.80×10^{-4}	4.50×10^{-4}	0.6900	-1.18×10^{-4}	4.92×10^{-5}	0.0166
UKB-a:32	Alcohol intake versus 10 years previously	-3.20×10^{-5}	2.10×10^{-4}	0.8770	-4.72×10^{-5}	2.28×10^{-5}	0.0380
UKB-a:46	Miserableness	4.70×10^{-5}	1.50×10^{-4}	0.7550	-4.41×10^{-5}	1.62×10^{-5}	0.0064
UKB-b:18835	Diagnoses - main ICD10: F20.0 Paranoid schizophrenia	1.70×10^{-6}	4.40×10^{-6}	0.7060	-9.52×10^{-7}	4.79×10^{-7}	0.0471
UKB-b:1464	Number of depression episodes	8.40×10^{-4}	5.40×10^{-4}	0.1250	-1.37×10^{-4}	5.95×10^{-5}	0.0212
UKB-b:3460	Alcohol intake versus 10 years previously	-8.90×10^{-5}	1.80×10^{-4}	0.6230	-3.88×10^{-5}	1.97×10^{-5}	0.0488
UKB-b:2047	Age started smoking in former smokers	-1.40×10^{-4}	4.40×10^{-4}	0.7540	9.82×10^{-5}	4.74×10^{-5}	0.0381
UKB-b:8993	Diagnoses - main ICD10: Q85.0 Neurofibromatosis (nonmalignant)	1.00×10^{-6}	2.20×10^{-6}	0.6510	-5.41×10^{-7}	2.43×10^{-7}	0.0263
UKB-b:11446	Diagnoses - secondary ICD9: E8788 Abn. reaction to other spec. surg op./ procedure without misadvent at time	4.10×10^{-6}	4.00×10^{-6}	0.3090	-8.74×10^{-7}	4.36×10^{-7}	0.0450
UKB-b:6244	Exposure to tobacco smoke outside home	1.20×10^{-4}	1.60×10^{-4}	0.4510	-3.51×10^{-5}	1.75×10^{-5}	0.0444
UKB-b:5779	Alcohol intake frequency.	-1.80×10^{-5}	4.00×10^{-4}	0.9640	-9.10×10^{-5}	4.34×10^{-5}	0.0358
UKB-a:244	Frequency of tenseness / restlessness in last 2 weeks	-1.50×10^{-4}	1.80×10^{-4}	0.3910	-4.32×10^{-5}	1.94×10^{-5}	0.0258
UKB-b:4454	Diagnoses - secondary ICD10: F42.9 Obsessive-compulsive disorder, unspecified	1.80×10^{-6}	2.80×10^{-6}	0.5190	-6.54×10^{-7}	3.01×10^{-7}	0.0297
UKB-b:18994	Miserableness	1.60×10^{-5}	1.30×10^{-4}	0.9020	$^{-}3.03 \times 10^{-5}$	1.44×10^{-5}	0.0358
UKB-b:1774	Diagnoses – main ICD10: G40.1 Localisation-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures	1.60×10^{-6}	2.10×10^{-6}	0.4610	4.67×10^{-7}	2.33×10^{-7}	0.0445
UKB-b:10316	Diagnoses – secondary ICD9: 3050 Nondependent abuse of alcohol	5.10×10^{-6}	3.80×10^{-6}	0.1860	8.35×10^{-7}	4.19×10^{-7}	0.0463

Table 2. List of the brain-related diseases or traits identified by polygenetic risk scores analysis.

	UK Biobank			
Ourcome	$P_{UK\text{-}female}$	$P_{UK\text{-}male}$	P_{UK}	$P_{2\text{-}MR}$
Worry too long after embarrassment	2.28×10^{-5}	0.0388	7.58×10^{-6}	0.0004
Suffer from 'nerves'	0.348	0.0929	0.0477	0.0062
Neuroticism score	0.0063	0.1142	0.0024	0.0018
Nervous feelings	0.0115	0.1670	0.0043	0.0158
Worrier / anxious feelings	0.0196	0.2930	0.0166	0.0013
Fluid intelligence score	0.0446	0.7114	0.0938	0.0269
Alcohol intake frequency	0.293	0.907	0.471	0.0166

variables of gut microbiota. We conducted this analysis in female and male, respectively. All of these statistical analyses were conducted by R (version 3.5.1). $p < 0.05$ was selected as the significant level.

Results

Discovery two-sample MR study

In total, 515 complex traits or diseases were selected as outcome to detect their relationships with gut microbiota. 41 of 515 complex traits or diseases were identified to be associated with gut microbiota (Table 1). All two-sample MR analysis results are shown in Supplementary Table 4.

We found that several psychiatric disorders or traits were associated with gut microbiota, such as worry too long after embarrassment (UKB-a:53, $P_{2\text{-}MR} = 0.0004$, $P_{HP} = 0.8950$) and worrier/anxious feelings (UKB-a:51, $P_{2\text{-}MR} = 0.0013$, $P_{HP} = 0.6720$), Nervous feelings (UKB-a:50, $P_{2\text{-}MR} = 0.0158$, $P_{HP} = 0.9190$) and Number of depression episodes (UKB-b:1464, $P_{2\text{-}MR} = 0.0212$, $P_{HP} = 0.1250$).

Additionally, we observed association signals between gut microbiota and neurological disorders, such as Neuroticism score (UKB-a:230, $P_{2\text{-}MR} = 0.0018$, $P_{HP} = 0.4840$), Autonomic neuropathy in endocrine and metabolic diseases (UKB-b:18910, $P_{2\text{-}MR} = 0.0086$, $P_{HP} = 0.0839$) and Neurofibromatosis (nonmalignant) (UKB-b:8993, $P_{2\text{-}MR} = 0.0263$, $P_{HP} = 0.6510$).

For others diseases or traits related to brain function, the most significant signals associated with gut microbiota were smoking and alcohol, such as alcoholic hepatic failure (UKB-b:6787, $P_{2\text{-}MR} = 0.0023$, $P_{HP} = 0.0785$), exposure to tobacco smoke outside home (UKB-a:20, $P_{2\text{-}MR} = 0.0138$, $P_{HP} = 0.1200$), smoking/smokers in household (UKB-a:18, $P_{2\text{-}MR} = 0.0126$, $P_{HP} = 0.5060$) and alcohol intake frequency (UKB-a:25, $P_{2\text{-}MR} = 0.0166$, $P_{HP} = 0.6900$).

Replication PRS analysis using UK biobank samples

Using UK Biobank samples, we calculated PRS of gut microbiota associated SNPs of each individual to validate the associations detected in discovery stage. After Pearson correlation and logistic regression analysis, five significant associations were validated in UK Biobank total samples and female samples, such as Neuroticism score ($P_{UK} = 0.0024$, $P_{UK\text{-}female} = 0.0063$, $P_{UK\text{-}male} = 0.1142$), Nervous feelings ($P_{UK} = 0.0043$, $P_{UK\text{-}female} = 0.0115$, $P_{UK\text{-}male} = 0.1670$) and Worrier/anxious feelings ($P_{UK} = 0.0166$, $P_{UK\text{-}female} = 0.0196$, $P_{UK\text{-}male} = 0.2930$) (Table 2).

Discussion

In this study, we performed two-sample MR analysis with gut microbiota as exposure and 515 complex diseases or traits as outcome. Intriguingly, 41 of 515 brain-related diseases or traits were detected to be significantly associated with gut microbiota. Furthermore, 5 of 41 were validated via conducting PRS analysis of the UK Biobank cohort. In this study, the most interesting finding is that associations between brain and gut microbiota existed sex differences. Besides, our study provided a novel perspective to understand the functional relevance of gut microbiota with brain-related diseases or traits.

Neuroticism was found to be significant in two-sample MR analysis ($P_{2\text{-}MR} = 0.0018$), and was validated in the UK biobank study ($P_{UK} = 0.0024$). A cross-sectional study reported that diversity and composition of gut microbiota were significantly correlated with personality, which was verified by 16S rRNA sequencing of gut microbiota in 672 subjects (male: 412, female: 272) (Kim et al. 2018). Specifically, neuroticism had a significant correlation with the abundance of Gammaproteobacteria ($r = 0.006$, $q = 0.045$) and Proteobacteria ($r = 0.007$, $q = 0.014$), when covariates (age, sex, BMI and nutrient intake) were controlled (Kim et al. 2018). The previous research suggested that neuroticism was associated with the alterations of Hypothalamic–Pituitary–Adrenal (HPA) stress response axis and the high circulating levels of inflammatory markers, such as IL-6 and C-reactive protein (Mangold and Wand 2006; Sutin et al. 2010). In addition, for Gram-negative gut commensal bacteria, bacterial translocation with immune responses to lipopolysaccharide (LPS) may play an important role in the pathophysiology of chronic inflammation (Maes et al. 2012). Besides, Neuropeptide Y is a pleiotropic gene implicated in neuroticism and is the important neural and

endocrine messengers in orchestrating microbiota-gut-brain axis in health and disease (Holzer and Farzi 2014; Melas et al. 2018). These research and results suggested that gut microbiota has a correlation with neuroticism through microbiota-gut-brain axis and immune system.

We identified that four mental health related traits showed significant associations with gut microbiota in both discovery and replication study, including Worry too long after embarrassment ($P_{2\text{-}MR} = 0.0004$, $P_{UK} = 7.58 \times 10^{-6}$), Suffer from 'nerves' ($P_{2\text{-}MR} = 0.0062$, $P_{UK} = 0.0477$), Nervous feelings ($P_{2\text{-}MR} = 0.0158$, $P_{UK} = 0.0043$) and Worrier/anxious feelings ($P_{2\text{-}MR} = 0.0013$, $P_{UK} = 0.0166$). Gut microbiota was a regulator of anxiety, and gut-derived peptides, such as neuropeptide Y and peptide YY, played a vital role in gut–microbiome–brain axis (Lach et al. 2018). More than half of the studies showed that regulation of gut microbiota was positive to improve anxiety symptoms, and non-probiotic interventions were more effective than probiotic interventions (Yang et al. 2019). HPA axis was recognised as the core stress efferent axis, which could coordinate any kind stressors acting on body or organism (Joseph and Golden 2017). Cortisol could have an effect on regulating stress of organs, including brain. The research indicated that brain could release corticotropin-releasing factor (CRF) with the effect of mental stress to increase the frequency of cecocolonic spike-burst activity (Golden et al. 2011). Therefore, both neural and hormonal lines of communication combine to influence emotions and gut microbiota through microbiota-gut-brain axis.

Moreover, depression episode is one of common symptoms nowadays, and was discovered to be significant ($P_{2\text{-}MR} = 0.0403$). Several previous studies found that gut microbiota greatly affected depression through immune system (Evrensel and Ceylan 2015; Valles-Colomer et al. 2019). Bacteroidales family was discovered to be associated with depression, through 16S rRNA gene sequence analysis of 37 patients and 18 non-depressed controls (Naseribafrouei et al. 2014). Based on emerging evidence, we suggested that gut microbiota is linked to depression.

Interestingly, according to PRS results, we found that gut microbiota contributed more to brain-related diseases or traits in females than in males. In PRS results, four brain-related traits were significant in female, but not significant in male. Epidemiological evidence revealed that female had a more significant correlation with stress related disorders compared with male (Bangasser and Valentino 2014). Gut microbiota had an influence on brain via gut-microbiota-brain axis, and there existed sex differences throughout lifespan (Jašarević et al. 2016). Consistent with this view, a matched cases–controls research used 16S rRNA gene-sequencing analysis to access sex-specific gut microbiota (Chen et al. 2018). They found that *Actinobacteria* level increased in female MDD patients and *Bacteroidetes* level decreased in male MDD patients, compared with healthy counterparts (Chen et al. 2018). Likewise, the changes of gut microbiota were induced by stress, which may affect mental health in a sex-specific way (Audet 2019). These emerging evidences suggested that there existed sex differences in gut microbiota in brain-related disease through gut-microbiota-brain axis.

Nowadays, gut microbiota attracted attention of many researchers. In this study, we systemically scanned 515 brain-related diseases or traits to explore the relationship with gut microbiota using two-MR analysis. Interestingly, we identified 41 significant traits or diseases associated with gut microbiota. More importantly, we replicated 5 significant results in the UK Biobank cohort, which was composed of ~500,000 individuals. Besides, we found that female has a more tendency to obtain brain-related diseases through influencing gut microbiota than male. Collectively, these findings indicated that modulating gut microbiota could be served as a new sight to investigate mechanism of complex brain-related diseases.

Nevertheless, there are some limitations in our study should be noted. First, due to the limited GWAS datasets of gut microbiota available online, it may influence accuracy of the results, although we have performed a replication stage to reduce chance of making mistakes. Besides, for gut microbiota, the detailed procedure of GWAS could not be performed as we wanted, which also had influence on the accuracy of gut microbiota related SNPs. The further functional analysis is needed to validate our findings in our future studies. Two-sample MR approach cannot figure out the possibility that the associations discovered are pleiotropic rather than causal, and even then, one cannot not fully discard the possibility of reverse causation. Therefore, we should seriously explain the associations of gut microbiota and brain-related diseases and traits.

Conclusions

In summary, our study successfully explored the associations between gut microbiota and brain-related diseases or traits by means of two-sample MR analysis and PRS analysis to UK Biobank study. We found there

existed a significant relationship between gut microbiota and brain, and the associations existed sex differences. Based on our results, we suggested that modulating the composition of gut microbiota and sex differences could be as a new sight to research genetic mechanism of brain-related diseases or traits.

Acknowledgements

We thank the UK Biobank participants and the UK Biobank team for their work in collecting, processing and disseminating these data for analysis. The UK Biobank study was approved by the National Health Service National Research Ethics Service (11/NW/0382). Our data dictionary ID for application is 46478.

Statement of interest

The authors have stated that they have no conflict of interest.

Author contributions

Xin Qi and Fanglin Guan drafted the manuscript. Feng Zhang designed the study. Xin Qi, Zhang, Fanglin Guan, Shiqiang Cheng, Yan Wen and Li Liu performed the statistical analyses. Mei Ma, Bolun Cheng, Chujun Liang, Xiao Liang, Lu Zhang, Ping Li, Xiaomeng Chu, Jing Ye, and Yao Yao provided feasible advice on data analysis and drafting manuscript. All authors read and approved the final manuscript. All authors discussed the results and commented on the manuscript.

Funding

This study is supported by the National Natural Scientific Foundation of China [81472925, 81673112, 81703177], the Key projects of international cooperation among governments in scientific and technological innovation [2016YFE0119100], the Natural Science Basic Research Plan in Shaanxi Province of China [2017JZ024], and the Fundamental Research Funds for the Central Universities.

ORCID

Bolun Cheng http://orcid.org/0000-0003-2915-5535
Chujun Liang http://orcid.org/0000-0003-4469-3171

Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

References

Anttila V, Bulik-Sullivan B, Finucane HK, Walters RK, Bras J, Duncan L, Escott-Price V, Falcone GJ, Gormley P, Malik R, et al. 2018. Analysis of shared heritability in common disorders of the brain. Science. 360:eaap8757.

Audet MC. 2019. Stress-induced disturbances along the gut microbiota-immune-brain axis and implications for mental health: does sex matter? Front Neuroendocrinol. 54: 100772.

Band G, Marchini J. 2018. BGEN: a binary file format for imputed genotype and haplotype data. bioRxiv.308296.

Bangasser DA, Valentino RJ. 2014. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Front Neuroendocrinol. 35:303–319.

Blauwendraat C, Heilbron K, Vallerga CL, Bandres-Ciga S, von Coelln R, Pihlstrøm L, Simón-Sánchez J, Schulte C, Sharma M, Krohn L, et al., International Parkinson's Disease Genomics Consortium (IPDGC). 2019. Parkinson's disease age at onset genome-wide association study: defining heritability, genetic loci, and α-synuclein mechanisms. Mov Disord. 34:866–875.

Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, et al. 2018. The UK Biobank resource with deep phenotyping and genomic data. Nature. 562:203–209.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 7: 335–336.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 4:7.

Chen JJ, Zheng P, Liu YY, Zhong XG, Wang HY, Guo YJ, Xie P. 2018. Sex differences in gut microbiota in patients with major depressive disorder. Neuropsychiatr Dis Treat. 14: 647–655.

Choi Y, Lee Sun J, Spiller W, Jung KJ, Lee JY, Kimm H, Back JH, Lee S, Jee SH. 2020. Causal associations between serum bilirubin levels and decreased stroke risk. ATVB. 40: 437–445.

Clapp M, Aurora N, Herrera L, Bhatia M, Wilen E, Wakefield S. 2017. Gut microbiota's effect on mental health: the gut-brain axis. Clin Pract. 7:987.

Cong X, Xu W, Romisher R, Poveda S, Forte S, Starkweather A, Henderson WA. 2016. Gut microbiome and infant health: brain-gut-microbiota axis and host genetic factors. Yale J Biol Med. 89:299–308.

Cross-Disorder Group of the Psychiatric Genomics C, Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, Perlis RH, Mowry BJ, Thapar A, Goddard ME, et al., International Inflammatory Bowel Disease Genetics Consortium (IIBDGC). 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet. 45:984–994.

Evrensel A, Ceylan ME. 2015. The gut-brain axis: the missing link in depression. Clinical psychopharmacology and neuroscience: the official scientific journal of the Korean College of. Clin Psychopharmacol Neurosci. 13:239–244.

García-Santisteban I, Cilleros-Portet A, Moyua-Ormazabal E, Kurilshikov A, Zhernakova A, Garcia-Etxebarria K,

Fernandez-Jimenez N, Bilbao JR. 2020. A two-sample Mendelian randomization analysis investigates associations between gut microbiota and celiac disease. Nutrients. 12:1420.

Golden SH, Wand GS, Malhotra S, Kamel I, Horton K. 2011. Reliability of hypothalamic-pituitary-adrenal axis assessment methods for use in population-based studies. Eur J Epidemiol. 26:511–525.

Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE. 2016. Genetic determinants of the gut microbiome in UK twins. Cell Host Microbe. 19:731–743.

Han G, Sun J, Wang J, Bai Z, Song F, Lei H. 2014. Genomics in neurological disorders. Genomics Proteomics Bioinformatics. 12:156–163.

Heffner JL, Strawn JR, DelBello MP, Strakowski SM, Anthenelli RM. 2011. The co-occurrence of cigarette smoking and bipolar disorder: phenomenology and treatment considerations. Bipolar Disord. 13:439–453.

Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, et al. 2018. The MR-Base platform supports systematic causal inference across the human phenome. eLife. 7:e34408.

Hilker R, Helenius D, Fagerlund B, Skytthe A, Christensen K, Werge TM, Nordentoft M, Glenthøj B. 2018. Heritability of schizophrenia and schizophrenia spectrum based on the nationwide Danish twin register. Biol Psychiatry. 83: 492–498.

Holzer P, Farzi A. 2014. Neuropeptides and the microbiota-gut-brain axis. Adv Exp Med Biol. 817:195–219.

Horwitz T, Lam K, Chen Y, Xia Y, Liu C. 2019. A decade in psychiatric GWAS research. Mol Psychiatry. 24:378–389.

Howie BN, Donnelly P, Marchini J. 2009. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. 5: e1000529.

Jašarević E, Morrison Kathleen E, Bale Tracy L. 2016. Sex differences in the gut microbiome-brain axis across the lifespan. Philos Trans R Soc Lond B Biol Sci. 371:20150122.

Joseph JJ, Golden SH. 2017. Cortisol dysregulation: the bidirectional link between stress, depression, and type 2 diabetes mellitus. Ann N Y Acad Sci. 1391:20–34.

Kim HN, Yun Y, Ryu S, Chang Y, Kwon MJ, Cho J, Shin H, Kim HL. 2018. Correlation between gut microbiota and personality in adults: a cross-sectional study. Brain Behav Immun. 69:374–385.

Kujala UM, Palviainen T, Pesonen P, Waller K, SillanpÄÄ E, Niemel Ä M, Kangas M, VÄhÄ-YpyÄ H, SievÄnen H, Korpelainen R, et al. 2020. Polygenic risk scores and physical activity. Med Sci Sports Exerc. 52:1518–1524.

Lach G, Schellekens H, Dinan TG, Cryan JF. 2018. Anxiety, depression, and the microbiome: a role for gut peptides. Neurotherapeutics. 15:36–59.

Larvie M, Fischl B. 2016. Chapter 3 – Volumetric and fiber-tracing MRI methods for gray and white matter. In: Masdeu JC, González RG, editors. Handbook of clinical neurology. Amsterdam (Netherlands): Elsevier; p. 39–60.

Liu L, Wen Y, Zhang L, Xu P, Liang X, Du Y, Li P, He A, Fan Q, Hao J, et al. 2018. Assessing the associations of blood metabolites with osteoporosis: a Mendelian randomization study. J Clin Endocrinol Metab. 103:1850–1855.

MacDonald BK, Sander JWAS, Cockerell OC, Shorvon SD. 2000. The incidence and lifetime prevalence of neurological disorders in a prospective community-based study in the UK. Brain. 123:665–676.

Maes M, Kubera M, Leunis JC, Berk M. 2012. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. J Affect Disord. 141:55–62.

Mangold DL, Wand GS. 2006. Cortisol and adrenocorticotropic hormone responses to naloxone in subjects with high and low neuroticism. Biol Psychiatry. 60:850–855.

Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, Tyrer JP, Chen TH, Wang Q, Bolla MK, et al., NBCS Collaborators. 2019. Polygenic risk scores for prediction of breast cancer and breast cancer subtypes. Am J Hum Genet. 104:21–34.

McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. 2003. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. JAMA Psychiatry. 60:497–502.

McVean GA, Altshuler DM, Durbin RM, Abecasis GR, Bentley DR, Chakravarti A, Clark AG, Donnelly P, Eichler EE, Flicek P, et al., 1000 Genomes Project Consortium. 2012. An integrated map of genetic variation from 1,092 human genomes. Nature. 491:56–65.

Melas PA, Guban P, Rahman MS, Lavebratt C, Forsell Y. 2018. Neuropeptide Y, stressful life events and personality trait conscientiousness: preliminary associations from a Swedish longitudinal study. Psychiatry Res. 263:48–53.

Mühleisen TW, Leber M, Schulze TG, Strohmaier J, Degenhardt F, Treutlein J, Mattheisen M, Forstner AJ, Schumacher J, Breuer R, et al. 2014. Genome-wide association study reveals two new risk loci for bipolar disorder. Nat Commun. 5:3339.

Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linløkken A, Wilson R, Rudi K. 2014. Correlation between the human fecal microbiota and depression. Neurogastroenterol Motil. 26:1155–1162.

O'Connell J, Sharp K, Shrine N, Wain L, Hall I, Tobin M, Zagury JF, Delaneau O, Marchini J. 2016. Haplotype estimation for biobank-scale data sets. Nat Genet. 48: 817–820.

OWH. 2010. Neurological disorders: public health challenges. J Policy Pract Intellect Disabil. 5:75–75.

Sagar R, Dandona R, Gururaj G, Dhaliwal RS, Singh A, Ferrari A, Dua T, Ganguli A, Varghese M, Chakma JK, et al. 2020. The burden of mental disorders across the states of India: the Global Burden of Disease Study 1990–2017. Lancet Psychiatry. 7:148–161.

Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, Võsa U, Mujagic Z, Masclee AAM, Jonkers DMAE, Oosting M, et al. 2019. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet. 51:600–605.

Schmitt A, Malchow B, Hasan A, Falkai P. 2014. The impact of environmental factors in severe psychiatric disorders. Front Neurosci. 8:19.

Steel Z, Marnane C, Iranpour C, Chey T, Jackson JW, Patel V, Silove D. 2014. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980-2013. Int J Epidemiol. 43:476–493.

Sullivan PF, Kendler KS, Neale MC. 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 60:1187–1192.

Sutin AR, Terracciano A, Deiana B, Naitza S, Ferrucci L, Uda M, Schlessinger D, Costa PT. 2010. High neuroticism and low conscientiousness are associated with interleukin-6. Psychol Med. 40:1485–1493.

Turpin W, Espin-Garcia O, Xu W, Silverberg MS, Kevans D, Smith MI, Guttman DS, Griffiths A, Panaccione R, Otley A, et al., GEM Project Research Consortium. 2016. Association of host genome with intestinal microbial composition in a large healthy cohort. Nat Genet. 48:1413–1417.

The UK Biobank Array Design Group. 2014. UK Biobank Axiom array: content summary. http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/UK-Biobank-Axiom-Array-ContentSummary-2014.pdf

Ursell LK, Metcalf JL, Parfrey LW, Knight R. 2012. Defining the human microbiome. Nutr Rev. 70(Suppl 1):S38–S44.

Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, Schiweck C, Kurilshikov A, Joossens M, Wijmenga C, et al. 2019. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol. 4:623–632.

Wain LV, Shrine N, Miller S, Jackson VE, Ntalla I, Artigas MS, Billington CK, Kheirallah AK, Allen R, Cook JP, et al. 2015. Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. Lancet Respir Med. 3:769–781.

Walters RK, Polimanti R, Johnson EC, McClintick JN, Adams MJ, Adkins AE, Aliev F, Bacanu SA, Batzler A, Bertelsen S, et al., 23andMe Research Team. 2018. Transancestral GWAS of alcohol dependence reveals common genetic underpinnings with psychiatric disorders. Nat Neurosci. 21:1656–1669.

Wang H, Braun C, Murphy E, Enck P. 2019. *Bifidobacterium longum* 1714^{TM} strain modulates brain activity of healthy volunteers during social stress. Am J Gastroenterol. 114: 1152–1162.

Yang B, Wei J, Ju P, Chen J. 2019. Effects of regulating intestinal microbiota on anxiety symptoms: a systematic review. Gen Psychiatr. 32:e100056.

Zhou X, Stephens M. 2012. Genome-wide efficient mixed-model analysis for association studies. Nat Genet. 44: 821–824.