Investigating Casual Associations among Gut Microbiota, Metabolites and Neurodegenerative Diseases: A Mendelian Randomization Study

Jin-Tai Yu (jintai_yu@fudan.edu.cn)
Huashan Hospital, Fudan University https://orcid.org/0000-0002-7686-0547
Jing Ning
Huashan Hospital Fudan University
Shu-Yi Huang
Huashan Hospital Fudan University
Shi-Dong Chen
Huashan Hospital Fudan University
Yu-Xiang Yang
Huashan Hospital Fudan University
Qiang Dong
Huashan Hospital Fudan University

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Abstract

Background

Recent studies had explored that the gut microbiota was associated with neurodegenerative diseases (including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS)) through the gut-brain axis, among which metabolic pathways played an important role. However, the underlying causality remained unclear. Our study aimed to evaluate potential causal relationships between gut microbiota, metabolites and neurodegenerative diseases through Mendelian randomization (MR) approach.

Methods

We selected genetic variants associated with gut microbiota traits (N = 18340) and gut microbiota-derived metabolites (N = 7824) from genome-wide association studies (GWASs). Summary statistics of neurodegenerative diseases were obtained from IGAP (AD: 17008 cases; 37154 controls), IPDGC (PD: 37 688 cases; 141779 controls) and IALSC (ALS: 20806 cases; 59804 controls) respectively.

Results

A total of 19 gut microbiota traits were found to be causally associated with risk of neurodegenerative diseases, including 1 phylum, 2 classes, 2 orders, 2 families and 12 genera. We found genetically predicted greater abundance of *Ruminococcus*, at genus level (OR:1.245, 95%CI:1.103,1.405; P = 0.0004) was significantly related to higher risk of ALS. We also found suggestive association between 12 gut microbiome-dependent metabolites and neurodegenerative diseases. For serotonin pathway, our results revealed serotonin as protective factor of PD, and kynurenine as risk factor of ALS. Besides, reduction of glutamine was found causally associated with occurrence of AD.

Conclusions

Our study firstly applied a two-sample MR approach to detect causal relationships among gut microbiota, gut metabolites and the risk of AD, PD and ALS, and we revealed several causal relationships. These findings may provide new targets for treatment of these neurodegenerative diseases, and may offer valuable insights for further researches on the underlying mechanisms.

Background

Neurodegenerative diseases are characterized by progressive loss of structure or function of neurons in the central or peripheral nervous system, which involves irreversible long-term motor or cognitive impairments[1]. The prevalence of neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), are rising worldwide with the increasing life expectancy. In recent years, emerging evidence has indicated that gut microbiota derived metabolites including short-chain fatty acids(SCFAs)[2, 3] and neurotransmitters such as glutamate[4], serotonin[5, 6] and γ-aminobutyric acid (GABA)[7] may play a central role in the gut-brain axis alterations and risk of neurodegenerative diseases [8]. However, few consistent links connecting gut microbiota and diseases or their associated metabolic pathways were found.

Increasing number of cross-sectional studies have implicated the association between gut microbiota and neurodegenerative diseases, including AD, PD, ALS [9]; however, such associations differed across studies. For example, an observational study(n = 25) found a significantly decreased abundance of Ruminococcaceae, and Actinobacteria and significant increase in abundance of Bacteroidetes in patients with Alzheimer's disease compared with control individuals[10]; while another cross-sectional study(n = 43) showed an opposite outcome of those microbiota[11]. Similarly, the association between gut microbiota and PD [12, 13] or ALS [14, 15] also differed in different studies. The results of those small observational studies should be considered with caution due to participant selection bias, confounding bias and reverse causation. However, it is crucial to identify whether those relationships were robust causal associations or spurious correlations.

Mendelian randomization (MR) approach, which uses genetic variants as instrumental variables(IVs), has been widely accepted to determine the causal effect of exposures on diseases[16]. Due to the random allocation of single nucleotide polymorphisms (SNPs) which is independent of confounders, MR is similar to randomized controlled trial and circumvent the limitations of previous observational studies.

Therefore, our study firstly applied a two-sample MR approach to detect causal relationships among gut microbiota, metabolites, and neurodegenerative disorders including AD, PD and ALS, using summary statistics from the largest genome-wide association studies (GWASs) so far.

Methods

Data sources and instruments

Summary statistics applied for investigating traits had the largest sample sizes, similar populations and with least sample overlap. Details of the contributing GWAS consortiums were listed in Additional File 1: Table S1.

Gut microbiota

We leveraged summary statistics from most comprehensive exploration of genetic influences on human gut microbiota so far. The MiBioGen consortium recruited 18,340 participants of multiple ancestries (including European, American Hispanic/Latin, East Asian and etc.) from 24 cohorts [17]. After extracting DNA from fecal samples, 16S rRNA gene sequencing was utilized to characterize the gut microbiome using SILVA[18] as a reference database, with truncation of the taxonomic resolution to genus level.

Gut metabolites

Considering the important roles of gut metabolites in microbiota-host crosstalk, we also leveraged summary-level data from a GWAS of the human metabolome conducted among European-descent subjects (TwinsUK and KORA, n=7824). The GWAS tested all 486 metabolite concentrations present in both datasets at each SNP. Then we applied HMDB[19] to obtain a list of 81 gut microbiota derived metabolite traits from all the quantified metabolites in the GWAS.

Neurodegenerative Diseases

We utilized the GWAS summary statistics from the largest and most recent datasets for AD, PD and ALS so far. We obtained the corresponding genetic variants from the International Genomics of Alzheimer's Project (IGAP) including 17,008 cases and 37,154 controls[20], the International Parkinson's Disease Genomics Consortium (IPDGC) including 37 688 cases and 141779 million controls[21], and the International Amyotrophic Lateral Sclerosis Genomics Consortium including 20,806 cases with ALS and 59,804 controls[22]. Cases of those neurodegenerative diseases were all clinically confirmed using published criteria.

Ethical approval for each study had been obtained in all original articles[17, 20-23], and no ethical approval for the current analyses was needed as they were based on publicly available summary statistics.

Selection of instrumental variables

To ensure the validity of the instrumental variables included for MR analyses, our study selected SNPs at thresholds for suggestive genome-wide significance ($P < 1 \times 10^{-5}$) as independent instruments for exposure (gut microbiota and metabolite traits). We manually checked all the identified SNPs by PhenoScanner GWAS database (http://www.phenoscanner.medschl.cam.ac.uk/) and excluded variants for the linkage disequilibrium (LDlink: https://ldlink.nci.nih.gov/, LD, R2 < 0.001), and all GWAS were assumed to be coded on the forward strand. We also computed the F-statistic of each exposure, and SNPs that had F-statistics less than 10 were excluded to avoid week instrument bias [38]. Finally, for gut microbiota instruments, a total of 8269 host SNPs were identified, which were associated with 200 gut microbiota traits (9phyla + 16 classes + 20 orders + 36 families + 119 genera), and for gut metabolite instruments, 3134 SNPs associated with 81 traits were included in our study. Summary statistics of these significant SNPs were assessed through Additional File 1: Table S2-S3.

Statistical analyses

We applied two sample MR as our main statistical methods to estimate causal associations between each instrument-exposure (gut microbiota and metabolite) and instrument-outcome (AD, PD and ALS). The MR approach was based on 3 key assumptions: (1) the genetic variant must be truly associated with the exposure; (2) the genetic variant should not be associated with confounders of the exposure-outcome relationship; (3) the genetic variant should only be related to the outcome of interest through the exposure under study[24].

Primary analyses were performed using Inverse-variance weighted (IVW) method, which essentially assumed the intercept was zero, and our results were corrected for multiple hypothesis testing using the Benjamini and Hochberg false discovery rate (FDR), as significance threshold was set at FDR-corrected p-values <0.05[25], while associations with P < 0.05, but not reaching the FDR-controlled threshold were reported as suggestive of association. Power calculations were conducted based on the website http://cnsgenomics.com/shiny/mRnd/[26] (see Additional File 1: Table S6).

To validate assumption 3 and improve the robustness of the findings, we also undertook a series of sensitivity analyses including MR-Egger regression, weighted mode, weighted median, simple median methods and robust adjusted profile score (MR.RAPS) method, which provided different assumptions about horizontal pleiotropy [27, 28]. However, MR-Egger method had the lowest power among the 6 methods, and was based on the instrument strength independent of the direct effects (INSIDE) assumption, with no measurement error in the SNP exposure effects (NOME) assumption[29]. Therefore, MR Egger was performed when I2GX was >0.9[30].

Cochran Q statistic and leave-one-out sensitivity analysis were also adopted to the SNPs that may influence the outcome through an unaccounted causal pathway, and Steiger analysis was performed to explore direction of causal effects[31]. Furthermore, MR-Egger intercept and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) global test were used to detect the presence of pleiotropy[32].

At last, we conducted multivariable MR (MVMR) analyses[33] using IVW method to estimate the direct and indirect effect of each exposure on an outcome, as we found a high degree of IV overlap across gut microbiota (Lentisphaerae at phylum level, Lentisphaeria at class level and Victivallales at order level) in univariable MR analyses on PD. Furthermore, we also conducted multivariable MR-Egger analyses to evaluate the horizontal pleiotropy for direct and indirect effects. The IVs used for MVMR analysis were listed in Additional File 1: Table S8.

The MR analyses were performed in the R version 4.0.2 computing environment using the latest TwoSampleMR (https://github.com/MRCIEU/TwoSampleMR), MVMR (https://github.com/WSpiller/MVMR), and MRPRESSO (https://github.com/rondolab/MR-PRESSO) packages.

Results

Associations between gut microbiota and neurodegenerative diseases

By the means of IVW method, results reaching a threshold of P < 0.05 are presented in Fig. 2. Causal effects were estimated by odds ratio (OR), which represented increase risk of binary outcomes (AD, PD, ALS) per SD increase in abundance of gut microbiota feature. By the means of IVW method, we found suggestive associations of host-genetic-driven increases in Actinobacteria at class level (OR, 1.027; 95%CI, 1.006–1.048; P = 0.013) ; Lactobacillaceae at family level (OR, 1.027; 95%CI, 1.006–1.048; P = 0.014); *Lachnoclostridium* at genus level (OR, 1.03; 95%CI, 1.005–1.056; P = 0.019) and higher risks of AD, while genetically increased in *Faecalibacterium* at genus level (OR, 0.975; 95%CI, 0.954–0.997; P = 0.028) were associated with protective effects on the risk of AD. We also found suggestive causal effect of *Ruminiclostridium6* at genus level (OR, 1.025; 95%CI, 1.006–1.045; P = 0.009) on higher risk of AD, while *Ruminiclostridium9* (OR, 0.969; 95%CI, 0.943–0.996; P = 0.009) on lower risk of AD. However, after calculating False Discovery Rate (FDR), we found that all q-values were over 0.05, suggesting no significant associations. What's more, associations between the gut microbiota traits and risk of AD were consistent in sensitivity analyses (see Table 1). MR- Egger intercept (we calculated I2GX, which were all over 0.9) and mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) were applied to test the directional pleiotropy, and all P values were over 0.05, suggesting no significant pleiotropy, while Cochran Q statistic of both the IVW test and the MR-Egger regression was used to test the heterogeneity, and no notable heterogeneity across instrument SNP effects was indicated (see Additional File 1: Table S7). However, we had limited power (less than 80%) to test causal effects of those gut microbiota features on AD.

Table 1
Sensitivity analyses of MR analyses of neurodegenerative diseases on gut metabolite features by MR Egger, simple mode, weighted me

Outcome	Exposure		Weighted mode		Weighted median		Simple mode		MR Egger
	Level	Microbiota	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI
AD	Class	Actinobacteria	1.048(1.009,1.088)	0.03	1.039(1.011,1.068)	0.01	1.022(0.979,1.067)	0.33	1.08(1.023
AD	Family	Lactobacillaceae	1.015(0.981,1.051)	0.41	1.022(0.996,1.049)	0.10	1.01(0.978,1.043)	0.56	0.986(0.93
AD	Genus	Faecalibacterium	0.98(0.946,1.015)	0.29	0.977(0.949,1.007)	0.13	0.979(0.942,1.018)	0.31	0.979(0.93
AD	Genus	Ruminiclostridium6	1.01(0.974,1.048)	0.59	1.019(0.992,1.048)	0.17	1.015(0.97,1.062)	0.53	1.005(0.95
AD	Genus	Ruminiclostridium9	0.984(0.926,1.045)	0.61	0.984(0.947,1.022)	0.40	0.983(0.921,1.05)	0.63	0.96(0.852
AD	Genus	Lachnoclostridium	1.004(0.939,1.074)	0.91	1.023(0.99,1.058)	0.17	1.006(0.944,1.073)	0.85	1.031(0.93
PD	Phylum	Lentisphaerae	0.745(0.555,0.999)	0.08	0.762(0.629,0.921)	0.01	0.743(0.538,1.026)	0.11	0.715(0.43
PD	Class	Lentisphaeria	0.751(0.559,1.009)	0.10	0.783(0.641,0.957)	0.02	0.747(0.539,1.037)	0.13	0.743(0.45
PD	Family	Oxalobacteraceae	1.202(0.934,1.547)	0.18	1.177(1.007,1.376)	0.04	1.194(0.901,1.583)	0.24	1.422(0.85
PD	Order	Victivallales	0.751(0.555,1.015)	0.11	0.783(0.64,0.959)	0.02	0.747(0.536,1.042)	0.13	0.743(0.45
PD	Order	Bacillales	1.221(0.931,1.601)	0.19	1.179(0.996,1.397)	0.06	1.215(0.934,1.581)	0.18	1.133(0.61
PD	Genus	Eubacteriumhalliigroup	1.462(0.97,2.202)	0.09	1.361(1.075,1.723)	0.01	1.526(0.967,2.407)	0.09	1.329(0.91
PD	Genus	Anaerostipes	0.588(0.336,1.029)	0.09	0.747(0.54,1.034)	0.08	0.6(0.342,1.052)	0.10	0.579(0.26
PD	Genus	Clostridiumsensustricto1	1.416(0.942,2.128)	0.15	1.413(1.043,1.915)	0.03	1.404(0.915,2.154)	0.17	1.728(1.00
ALS	Genus	RuminococcaceaeUCG004	1.259(0.92,1.723)	0.18	1.251(1.06,1.476)	0.05	1.263(0.934,1.707)	0.17	0.76(0.398
ALS	Genus	Lachnospira	1.15(0.745,1.776)	0.56	1.298(0.987,1.708)	0.01	1.431(0.933,2.196)	0.16	2.518(0.70
ALS	Genus	Fusicatenibacter	0.882(0.635,1.225)	0.46	0.838(0.703,0.999)	0.06	0.873(0.617,1.234)	0.16	0.702(0.42
ALS	Genus	Catenibacterium	0.924(0.757,1.128)	0.48	0.884(0.756,1.034)	0.05	0.927(0.731,1.176)	0.45	0.617(0.15
ALS	Genus	Ruminococcusgnavusgroup	0.811(0.628,1.047)	0.14	0.887(0.775,1.014)	0.12	0.776(0.599,1.004)	0.57	0.921(0.54

Abbreviations: OR = Odds ratios for associations of genetically predicted gut microbiota traits with neurodegenerative diseases; CI = confidence interval; MR = disease; PD = Parkinson's disease; ALS = Amyotrophic Lateral Sclerosis.

Causal relationship between gut microbiota and other neurodegenerative diseases were also analysized by the same process. Our study revealed that genetically increased abundance of Lentisphaerae at phylum level (OR, 0.836; 95%CI, 0.724,0.965; P = 0.015); Lentisphaeria at class level (OR, 0.847; 95%CI, 0.728–0.986; P = 0.032) and Victivallales at order level (OR, 0.847 ;95%CI, 0.728–0.986; P = 0.032) were potentially associated with a protective effect of PD. In contrast, no notable effects of the three gut microbiota features on the risk of PD could be observed after mutual adjustment using multivariable MR method (see Additional File 1: Table S7). In addition, genetically increased abundance of Oxalobacteraceae at family level (OR,1.13; 95%CI,1.003–1.273; P = 0.044); Bacillales at order level (OR, 1.144; 95%CI, 1.013–1.292; P = 0.03); *Eubacteriumhalliigroup* (OR, 1.253; 95%CI, 1.055,1.487; P = 0.01)) and *Clostridiumsensustricto1* (OR, 1.354; 95%CI, 1.068–1.716; P = 0.012)) were related to higher risk of AD; while *Anaerostipes* (OR, 0.744;95%CI, 0.587–0.944; P = 0.015) was related to protective effect of PD (Fig. 2).

Besides, genetically increased *Lachnospira* (OR, 1.315; 95%CI, 1.063–1.628; P = 0.012); decreased *Fusicatenibacter* (OR, 0.855; 95%CI, 0.752,0.972; P = 0.016) and *Catenibacterium* (OR, 0.848;95%CI, 0.74–0.97; P = 0.017) were potentially related to a higher risk of ALS. Our study also revealed that increased *RuminococcaceaeUCG004* (OR, 1.245(1.103–1.405); 95%CI, 1.103–1.405; P = 0.0004) and decreased *Ruminococcusgnavusgroup* (OR, 0.884; 95%CI,

0.792,0.986; P = 0.027) were related to a higher risk of ALS. Among all those results, we found a significant causal effect of increased *RuminococcaceaeUCG004* on risk of ALS (FDR-corrected P-value < 0.05) (Fig. 2).

Those estimate effects mentioned above were considered robust (Table 1) with no directional pleiotropy or heterogeneity was significant (see Additional File 1: Table S7), and MR power calculation results were showed in Additional File 1: Table S6.

Associations Between Gut Metabolites And Neurodegenerative Diseases

Among 81 gut microbiota-derived metabolites incorporated in our MR analyses, we found 11 suggestive estimate effects of gut metabolite on neurodegenerative diseases. Those metabolites were classified into 2 types: host-derived or dietary molecules[34].

With regard to host metabolites transformation, our study suggested that increased abundance of taurodeoxycholate, which was a product of primary bile acids (OR, 1.16 for risk ratio of ALS per SD unit of taurodeoxycholate; 95%CI, 1-1.345; P = 0.050) was associated with higher risk of ALS. However, no Steroid hormone was proved relevance to neurodegenerative diseases.

For the dietary molecules, amino acids, complex plant polysaccharides and polyphenols were considered to exert impact on brain function. In tryptophan metabolism, our study revealed that serotonin (OR, 0.535; 95%CI, 0.292–0.979; P = 0.043) was a protection factor of PD, while kynurenine (OR, 1.756; 95%CI, 1.113–2.769; P = 0.015) was a risk factor of ALS. In arginine metabolism, dimethylarginine (OR, 1.826; 95%CI, 1.003–3.321; P = 0.049) was suggested to be related to higher risk of ALS. Phenylacetate, a modulator of central adrenergic functions (OR, 1.064; 95%CI, 1.008–1.124; P = 0.024). Other ammino acids such as glutamine (OR, 0.803; 95%CI, 0.667–0.968; P = 0.022) and isoleucine (OR,0.791; 95%CI, 0.678–0.923; P = 0.003) were revealed as protective factors of AD and PD respectively. Besides, phenylalanine (PAA), one of phenylalanine derivatives (OR, 1.064; 95%CI, 1.008–1.124; P = 0.024) was indicated to increase the risk of AD. What's more, we also found that hippurate, a product of polyphenols (OR, 1.531; 95%CI, 1.142–2.051; P = 0.004) was associated with high risk of ALS. Gut microbiota also generated a protective factor of AD, mannitol (OR, 0.993; 95%CI, 0.988–0.998; P = 0.009), and a risk factor of PD (OR, 2.143; 95%CI,1.026–4.476; P = 0.043).

What's more, those results were judged to be reliable without pleiotropy through sensitivity analyses (Table 2, Additional File 1: Table S7). However, no significant association was revealed (FDR-corrected P-values > 0.05), and MR power calculation results were showed in Additional File 1: Table S6.

Table 2
Sensitivity analyses of MR analyses of neurodegenerative diseases on gut metabolite features by MR Egger, simple mode, weighted median and

Outcome	Exposure	MR Egger		Simple mode		Weighted median		Weighted mode	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
AD	Mannitol	0.962(0.911,1.016)	0.19	1(0.946,1.058)	0.99	0.976(0.944,1.009)	0.15	0.981(0.935,1.029)	0.45
AD	Glutamine	0.617(0.441,0.864)	0.03	0.972(0.654,1.445)	0.89	0.727(0.573,0.923)	0.01	0.714(0.547,0.932)	0.04
AD	Phenylacetate	1.046(0.965,1.134)	0.31	1.058(0.946,1.184)	0.36	1.063(0.99,1.14)	0.09	1.063(0.989,1.142)	0.14
PD	Isoleucine	0.847(0.677,1.06)	0.19	0.758(0.517,1.11)	0.19	0.818(0.667,1.004)	0.06	0.819(0.679,0.987)	0.07
PD	Hydrocinnamate	2.296(0.914,5.772)	0.10	1.942(0.75,5.029)	0.19	2.026(1.092,3.761)	0.03	1.942(0.937,4.024)	0.10
PD	Serotonin	0.672(0.178,2.545)	0.57	0.418(0.11,1.595)	0.22	0.498(0.218,1.14)	0.10	0.459(0.15,1.404)	0.19
PD	Arabinose	1.939(0.379,9.935)	0.51	2.345(0.752,7.319)	0.24	2.156(0.862,5.39)	0.10	2.184(0.836,5.708)	0.21
ALS	Hippurate	1.2(0.611,2.357)	0.61	1.909(0.992,3.673)	0.07	1.405(0.963,2.051)	0.08	1.277(0.784,2.079)	0.34
ALS	Kynurenine	1.956(0.772,4.956)	0.17	1.791(0.567,5.654)	0.33	1.682(0.885,3.197)	0.11	1.791(0.859,3.733)	0.13
ALS	Dimethylarginine	1.525(0.262,8.872)	0.64	3.646(0.655,20.297)	0.15	2.202(0.913,5.315)	0.08	3.073(0.831,11.369)	0.10
ALS	Taurodeoxycholate	1.132(0.683,1.876)	0.64	1.125(0.783,1.616)	0.54	1.138(0.922,1.404)	0.23	1.158(0.833,1.608)	0.40

Abbreviations: OR = Odds ratios for associations of genetically predicted gut microbiota-derived metabolite traits with neurodegenerative diseases; CI = confic
Mendelian randomization; AD = Alzheimer's disease; PD = Parkinson's disease; ALS = Amyotrophic Lateral Sclerosis.

Discussion

In the present MR study, we found significant association of increased abundance of genera *RuminococcaceaeUCG004* and higher risk of ALS. Besides, we found suggestive evidence of causal associations of Actinobacteria, Lactobacillaceae, *Faecalibacterium, and Ruminiclostridium, Lachnoclostridium* with AD, of Lentisphaerae, Lentisphaeria, Oxalobacteraceae, Victivallales, Bacillales, *Eubacteriumhalliigroup, Anaerostipes, and Clostridiumsensustricto1* with PD, and of *Lachnospira, Fusicatenibacter, Catenibacterium, and Ruminococcusgnavusgroup* with ALS. What's more, metabolites including amino acids, bile acids, amino acids, polyphenols produced by gut microbiota were also potentially related to the risks of neurodegenerative disorders, indicating their important roles in gut microbiota–brain axis.

A previous MR study have suggested that increase in *Blautia* and elevated γ-aminobutyric acid (GABA) were related to lower risk of AD[35]. However, our study failed to repeat these findings, nor *Blautia* or GABA including putrescine, glutamate, arginine or ornithin which produces GABA were found related to risk of AD,

which is potentially due to lack of significance of results and scale of GWAS. Another MR study proved no causal association of trimethylamine N-oxide (TMAO) or its precursor with AD[36], which was consistent with our results. What's more, our finding of Actinobacteria at family level as a risk factor of AD was opposite to previous studies [10], while the findings of relationships between Lactobacillaceae, *Faecalibacterium* with AD[11] were in accordance with the result of previous cross-sectional studies. Interestingly, genera *Ruminiclostridium6 and Ruminiclostridium9* represent different effects on risk of AD in our analysis results, which remind us that inconsistencies in results of previous clinical studies were potentially due to insufficiently digging deeper into classification of genera level of gut microbiota. Besides, our study suggested that phenylacetate, which was a potential tracer of glibal metabolism was related to increased risk of AD[37]. In addition, mannitol, a microbial metabolite was found as protective factor of AD, which may provide new ideas for disease interventions.

What's more, our study revealed suggestive causal effect of increased abundance of phylum Lentisphaerae, class Lentisphaeria, order Victivallales on protective effects of PD, however, no direct effect revealed after multivariable MR analysis, while no relevant result was reported in previous studies either, therefore, such results should be treated with caution. Other associations of Family Oxalobacteraceae, Order Bacillales, *Eubacteriumhalliigroup, Anaerostipes and Clostridiumsensustrictol* with risk of PD were in accordance with the result of previous cross-sectional studies[12, 13, 38]. In a previous clinical study, which compared the fecal microbiota of 25 ALS patients with 32 controls, significant higher abundance of uncultured Ruminococcaceae at genus level was observed in ALS patients[14]. However, our study found significant association between *RuminococcaceaeUCG004* and higher risk of ALS, and suggestive association between *Ruminococcusgnavusgroup* and lower risk of ALS. Inconsistent results between these studies may likely be attributed to small study sample sizes of previous observational studies, sample heterogeneity, and different sequencing technologies. Therefore, a standardized classification system for gut microbiota at genus level or even more specific level is crucial to direct mechanism researches and provide more accurate clinical guidance.

Tryptophan is broken down by the microbiota into indole derivatives and also tryptamine and kynurenine metabolites, and those metabolites were considered important in gut-brain axis[39, 40]. Previous studies have revealed that glutamate signals are destroyed by serotonergic overdrive, and serotonergic dysfunction is associated with the development of motor and non-motor symptoms and complications in Parkinson's disease[41]. What's more, kynurenine Pathway (KP) of tryptophan degradation is involved with several neuropathological features present in ALS including neuroinflammation, excitotoxicity, oxidative stress, immune system activation and dysregulation of energy metabolism[42], previous clinical studies have revealed that serum kynurenine in control were lower than that in ALS[43]. Our study proved that serotonin was protective factor of PD, while kynurenine was risk factor of ALS, and those molecules may become potential biomarkers to assess the progression of relative diseases. In addition, other amino acid such as glutamine and isoleucine were found causally associated with lower risk of AD and PD. Actually, up to 50% of all α-amino groups of glutamate and glutamine are derived from leucine. Leucine is a regulator of the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1), which is critical on protein synthesis and degradation, autophagy as well as maintenance of glutamate homeostasis, and may have effects on the neuronal solute transport and the excitatory neurotransmitter function[44]. Moreover, in the glutamate-glutamine cycle, synaptically-released glutamate is rapidly transported into astrocytes, and glutamine is then released by astrocytes through SN-type glutamine transporters into the extracellular fluid. Aβ has been shown to reduce the surface expression of GLT-1and to impair astrocyte glutamate uptake[45, 46]. A recent study demonstrated that altered astrocyte glutamine synthesis directly impaired neuronal GABA synthesis in brain slices of the 5xFAD mouse model of AD[47], and our results provided clinical evidence to confirm that reduction of glutamine in peripheral blood was causally associated with occurrence of AD.

Bacterial metabolites produced from polyphenol precursors were also found at levels sufficient to exert biological effects enter circulation[48]. In vitro cultures have shown that polyphenol metabolites such as ferulic acid are able to exert protective effects on neuronal cultures and neurodegenerative models, mostly through a decrease in inflammatory responses[49, 50], however, in vivo evidence remains lacking. Our study suggested hippurate, belongs to the group of uremic toxins as a risk factor of ALS, which may indicate potential treatment of disease. Since those neurodegenerative diseases develop through a long prodromal phase, it is plausible that our findings may inform early interventions by targeting the microbiota via gut microbiota transplantation, psychobiotics, or antibiotics in the future.

Among the strengths of the study are the most comprehensive MR study on association of gut microbiota and metabolite traits with neurodegenerative diseases, and the largest sample size so far. However, our study still suffers from several limitations. Firstly, most of the results did not survive a strict FDR correction. However, MR was a hypothesis-driven approach, and it could be used to detect some causal relationships regardless of FDR adjusting when some biological evidence exists. Secondly, 16S rRNA gene sequencing describes gut microbiota from genus to phylum level only, and metagenomic and multiomic approaches may offer opportunities to target gut microbiota compositon at a more specific level, avoiding bias if species of more specific level associated with neurodegenerative diseases. Finally, gut microbiota is affected by several environmental factors including diet and lifestyle, whereas those confounders which were not available in present studies were hardly to be excluded.

Conclusions

In summary, gut microbiota plays a crucial role in normal development and maintenance of brain function. Our study first applied a MR study to reveal causal relationships between some specific gut microbiota, metabolites and risks of AD, PD or ALS. However, extensive additional works are still required to characterize the effects of the microbiota-gut-brain axis on neurodegenerative diseases, and to find potential treatments by altering gut microbiota compositions.

Abbreviations

AD: Alzheimer's disease; PD: Parkinson's disease; ALS: Amyotrophic lateral sclerosis; SCFAs: Short-chain fatty acids; FMT: Fecal microbiota transplantation; IV: Instrumental variable; IVW: Inverse variance weighted; GWAS: Genome-wide association study; SNPs: Single nucleotide polymorphisms; OR: Odds ratio; CI, confidence interval; FDR: False discovery rate; RAPS: Robust adjusted profile score; TMAO: Trimethylamine N-oxide;

Declarations

Ethics approval and consent to participate

This study is based on publicly available summarized data. Individual studies within each genome-wide association study received approval from a relevant institutional review board, and informed consent was obtained from participants or from a caregiver, legal guardian, or other proxy.

Consent for publication

Not applicable.

Availability of data and materials

The data used in this study were publicly available and can be accessed via the links described in the Acknowledgement and the references in the manuscript. The datasets supporting the conclusions of this article are included within the article and its additional files

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JTY had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: JTY. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: JN, SYH, SDC. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: JN and YXY. Obtained funding: JTY. Administrative, technical, or material support: JTY, and QD. All authors contributed to the writing and revisions of the paper and approved the final version.

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All data used in the current study were based on summary-level data that have been made publically available. Summary data from genome-wide association studies for the gut microbiota is available at https://www.mibiogen.org/(Kurilshikov et al.). Metabolomic GWAS summary statistics are available for download at metabolomics gwas server (helmholtz-muenchen.de (Shin et al.). Summary level data for AD (Jansen et al.) can be obtained from https://ctg.cncr.nl/ (Jansen et al.), for PD (Nalls et al.) from https://pdgenetics.org/resources (participants from 23andMe Inc. were excluded), and for ALS (Nicolas et al.) from http://als.umassmed.edu . All data generated or analysed during this study are included in this published article and its supplementary information files.

Authors' information

[1]Department of Neurology and Institute of Neurology, Huashan Hospital, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Shanghai Medical College, Fudan University, Shanghai, China.

Corresponding authors

Correspondence to Jin-Tai Yu (jintai_yu@fudan.edu.cn).

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Figures

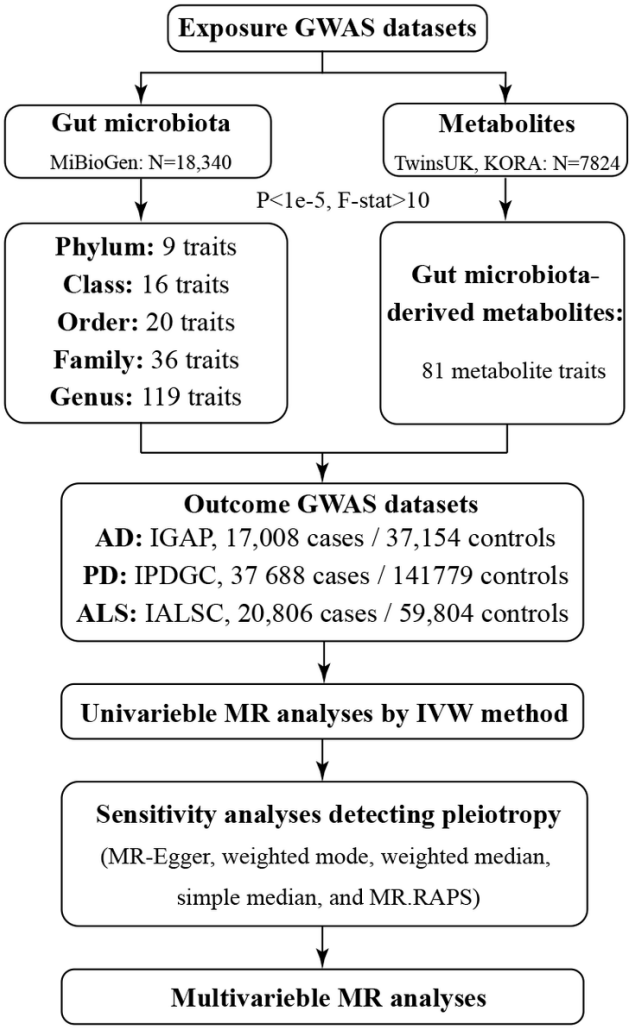

Figure 1

Flowchart of current study. AD: Alzheimer's disease; PD, Parkinson's disease; ALS, Amyotrophic lateral sclerosis.

Outcome	Level	Exposure	Number of SNPs	OR(95%CI)	P	FDR
AD	Class	Actinobacteria	15	1.027(1.006,1.048)	0.0127	0.203
AD	Family	Lactobacillaceae	9	1.027(1.006,1.05)	0.0135	0.486
AD	Genus	Faecalibacterium	10	0.975(0.954,0.997)	0.0277	3.296
AD	Genus	Ruminiclostridium6	16	1.025(1.006,1.045)	0.0092	0.547
AD	Genus	Ruminiclostridium9	9	0.969(0.943,0.996)	0.0266	1.055
AD	Genus	Lachnoclostridium	13	1.03(1.005,1.056)	0.019	0.565
PD	Phylum	Lentisphaerae	9	0.836(0.724,0.965)	0.0148	0.133
PD	Class	Lentisphaeria	8	0.847(0.728,0.986)	0.0324	0.518
PD	Order	Victivallales	8	0.847(0.728,0.986)	0.0324	0.648
PD	Order	Bacillales	9	1.144(1.013,1.292)	0.03	0.300
PD	Family	Oxalobacteraceae	14	1.13(1.003,1.273)	0.0439	1.580
PD	Genus	Eubacteriumhalliigroup	16	1.253(1.055,1.487)	0.0101	1.202
PD	Genus	Anaerostipes	13	0.744(0.587,0.944)	0.0147	0.875
PD	Genus	Clostridiumsensustricto1	7	1.354(1.068,1.716)	0.0123	0.488
ALS	Genus	RuminococcaceaeUCG004	11	1.245(1.103,1.405)	0.0004	0.048
ALS	Genus	Lachnospira	6	1.315(1.063,1.628)	0.0117	0.696
ALS	Genus	Fusicatenibacter	18	0.855(0.752,0.972)	0.0164	0.651
ALS	Genus	Catenibacterium	5	0.848(0.74,0.97)	0.0167	0.497
ALS	Genus	Ruminococcusgnavusgroup	12	0.884(0.792,0.986)	0.0272	0.647

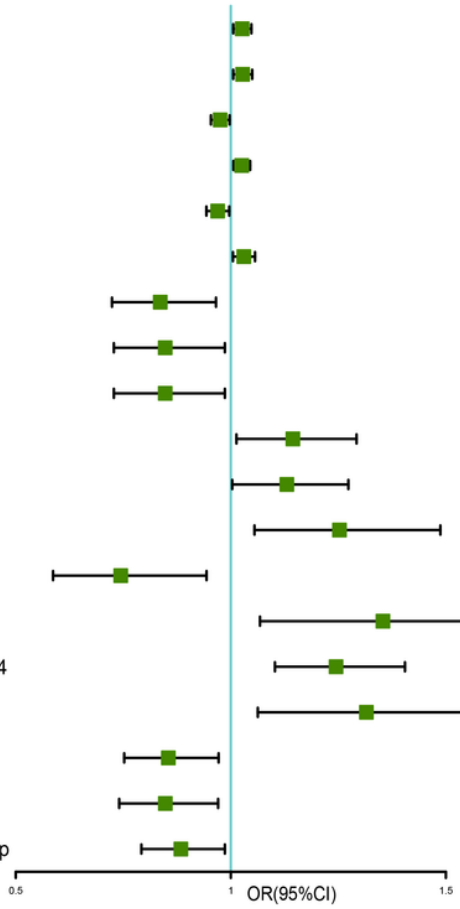

Figure 2

Associations of genetically predicted gut microbiota with risk of neurodegenerative diseases using IVW method. OR, odds ratio; CI, confidence interval; FDR: False discovery rate.

Outcome	Exposure	Number of SNPs	OR(95%CI)	P	FDR
AD	Mannitol	12	0.969(0.946,0.992)	0.009	0.698
AD	Glutamine	8	0.803(0.667,0.968)	0.022	0.876
AD	Phenylacetate	8	1.064(1.008,1.124)	0.024	0.655
PD	Isoleucine	10	0.791(0.678,0.923)	0.003	0.232
PD	Hydrocinnamate	14	1.674(1.033,2.712)	0.037	1.48
PD	Serotonin	15	0.535(0.292,0.979)	0.043	1.149
PD	Arabinose	4	2.143(1.026,4.476)	0.043	0.862
ALS	Hippurate	14	1.531(1.142,2.051)	0.004	0.352
ALS	Kynurenine	36	1.756(1.113,2.769)	0.015	0.627
ALS	Dimethylarginine	32	1.826(1.003,3.321)	0.049	1.315
ALS	Taurodeoxycholate	13	1.16(1,1.345)	0.05	1.007

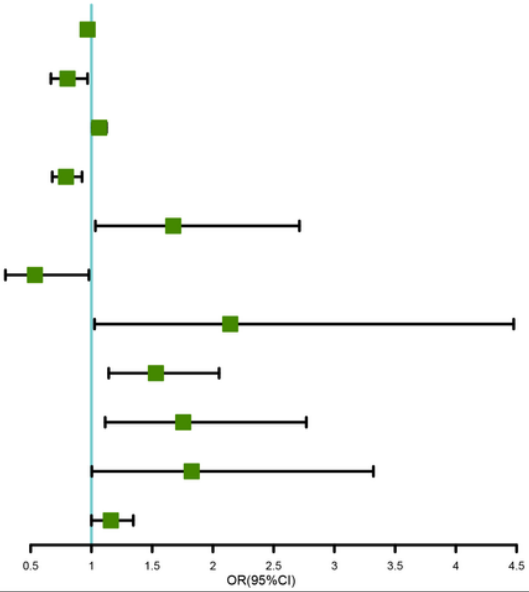

Figure 3

Associations of genetically predicted gut microbiota-dependent metabolites with risk of neurodegenerative diseases using IVW method. OR, odds ratio; CI, confidence interval; FDR: False discovery rate.

Supplementary Files

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