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Research Article

Gut Microbiota and Central Nervous System: A Bidirectional Two-Sample Mendelian Randomized Analysis

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ABSTRACT

Background: Previous studies have shown that alterations in the gut microbiota are associated with the progression of Central Nervous System (CNS) disorders. Whether this connection reflects a causal relationship still unclear. We aimed to reveal a causal relationship between the gut microbiota and CNS diseases such as Anoxic Brain Injury (ABI) and Bacterial Meningitis (BM).

Methods: A two-sample bi-directional Mendelian Randomization (MR) analysis was performed by using genetic variants from genome-wide association studies as instruments variables for gut microbiota, ABI and BM. This study used inverse variance weighted, weighted median, MR-Egger and weighted mode methods to evaluate the causal relationship among gut microbiota, ABI and BM. Sensitivity analyses including horizontal pleiotropy analysis, Cochran's Q test, and leave-one-out method were subsequently performed to assess the reliability of the results.

Results: We found that the increased abundance of *Lachnospiraceae* family and *Butyricoccus* genus was positively associated with the risk of ABI. The increased abundance of *Lactococcus*, *Ruminococcus gauvreauii* and Desulfovibrionales genera were positively associated with the risk of BM, while *Eubacterium ventriosum* genus, *Erysipelatoclostridium* genus and NB1n order were negatively associated with the risk of BM. On the other hand, CNS disorders altered the composition of the gut microbiota.

Conclusion: MR analysis has shown a bidirectional causal relationship between the abundance of specific bacteria and ABI and BM, providing evidence for gut microecological therapies for ABI and BM.

Keywords: Gut microbiota; Anoxic brain disease; Bacterial meningitis; Mendelian randomization; Single nucleotide polymorphism

INTRODUCTION

Anoxic Brain Injury (ABI), such as traumatic brain injury, stroke, cardiac arrest, asphyxia and neonatal ischemic hypoxic encephalopathy, is a common clinical cause of central nervous system injury [1-5]. It can occur in various age groups with poor prognosis. In severe cases may have permanent mental and cognitive dysfunction [1,6,7]. Bacterial Meningitis (BM) is an infectious disease of the CNS that commonly affects adults and children and with high morbidity, mortality and sequelae characteristics. Currently, the conservative treatments of ABI and BM have not yielded satisfactory therapeutic results.

The balance and stability of the gut microbiota is crucial for host healthy. In contrast, conditions such as hypoxia and infection will be disordering gut microbiota, disrupting the bidirectional balance between the gut and brain in Table 1 and causing cognitive and motor deficits [8,9]. Numerous studies have shown that the gut microbiota was involved in the regulation of cellular and molecular mechanisms of the brain injury process, found that a decreased diversity of intestinal flora in patients with CNS diseases, mainly in the abundance of Clostridium, *Anaerostipes*

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and *Lachnobacterium* [10]. In addition, research showed the correlation between the changed gut microbiota and clinical phenotype [11]. Meanwhile, it has been showed that changed flora are associated with the onset and progression of ABI and BM [5,10].

Seki, et al. found Klebsiella may as an outstanding predictor indicator of brain injury in preterm infants [12]. Animal study have shown that the prognosis of ABI in rats can be improved by intervening gut microbiota (such as butyrate-producing bacteria) [5,13]. However, the causal relationship still unclear.

The intestinal flora is the largest immune organ in the human body and its metabolites such as Short-Chain Fatty Acids (SCFA), tryptophan and kynurenine are involved in immune regulation in the body and exert anti-inflammatory effects [14,15]. In fact, due to the numerous confounding factors in clinical and animal studies, it is still difficult to clarify how gut microbiota connects with CNS diseases. The MR analysis method can exclude the interference of confounding factors and avoid the influence of reverse confounding factors, which makes the results more rigorous and reliable.

This study first to use a two-sample bidirectional MR method to analyze the potential causal relationship between the gut microbiota and two different CNS disorders (ABI and BM), as well as to investigate the genetic relationship between them.

MATERIALS AND METHODS

Study design

We conducted a two-sample bidirectional MR study to investigate the causal relationship between the gut microbiome and ABI and BM. To ensure valid Instrumental Variables (IVs) were obtained, MR was designed must base on three basic assumptions as follows: (1) Single Nucleotide Polymorphisms (SNPs) were robustly associated with exposure factors; (2) SNPs must be independent of any conventional and unknown confounders; (3) SNPs must be associated with outcomes only through exposure factors.

Source of datasets

Datasets of gut microbiota, ABI and BM were obtained from the GWAS database. The gut microbiota data was from a large GWAS analysis of 24 cohorts (18,340 individuals) conducted by MiBioGen consortium [8], which included genome-wide genotyping and 16sRNA sequencing data. ABI dataset (contains 191 cases and 205,799 controls) and BM dataset (contains 574 cases and 217,485 controls) were conducted by FinnGen consortium derived from European descent groups (Table 2).

This study performed the secondary analysis using public GWAS datasets and the Institutional Review Board review was not required.

Instrumental variables

SNPs were selected as IVs at a threshold of $P<1 \times 10^{-5}$. Meanwhile, we only selected independent genetic variants which are not in the linkage disequilibrium (defined as R2<0.001, Kb=10000). Then, SNPs that did not have A/T or C/G polymorphisms were excluded from the pool of SNPs based on the principle that the effects of selected SNPs on exposure and outcome were caused by having the same allele. We also calculated F-statistics for the SNPs to assess their instrumental strengths. F-statistic less than 10 were removed. Ultimately, 2037 SNPs were identified that were associated with 195 microbiota traits (9 phyla, 16 classes, 20 orders, 31 families, 119 genera). The study used two-sample MR analysis for causal analysis.

Mendelian randomization analysis

In this study, we choose Inverse Variance Weighted (IVW), MR-Egger regression, weighted median estimator and weighted mode for MR analysis. Their characteristics have been described by several studies [16-18].

In addition, outliers can be detected for pleiotropy bias through MR of pleiotropy residuals and Cochran's Q test to quantify the heterogeneity among the selected SNPs (P<0.05 was considered as possible heterogeneity in IVs) [19]. A leave-one-out sensitivity analysis was performed on the results by observing whether there was a statistical difference before and after removing each SNP. If there is little change in the results removing the SNP, which indicates that the SNP would not have a nonspecific effect on the effect estimate?

Evaluation of horizontal multidirectional and heterogeneity

The intercept term of MR-Egger regression detects the presence of directional heterogeneity, when the ending term egger intercept is close to zero; it represents no heterogeneity in the IVs. Analyses were conducted by the "TwoSampleMR" and "MendelR" packages [20,21]. Results were presented as Odds Ratios (OR) with respective 95% CI. All presented P-values were two-sided and statistical significance was set at the 5% level.

RESULTS

Genetic instruments for gut microbiome

There were 195 bacteria traits containing five biological levels in our study. The detailed information of the SNPs for each bacteria trait.

Gut microbiota exposure was obtained from 24 cohort studies in the United States, Canada, Israel, South Korea, Germany, Denmark, The Netherlands, Belgium, Sweden, Finland and the United Kingdom. After removing linkage disequilibrium, a total of 2037 SNPs were enrolled. In addition, we collected additional information about the SNPs, such as effector alleles, beta, se and P values.

Mendelian randomization analysis of gut microbiota and ABI and BM

Based on several different MR methods, we observed a potential causal relationship between the gut microbiota and ABI and BM. With the result in IVW, we found two gut taxa positively associated with risk of ABI (family_*Lachnospiraceae*: OR 5.13, 95%CI 1.13-23.32; genus_*Butyricicoccus*: OR 6.53,95%CI 1.47-29.01). While three gut taxa positively associated with BM risk (genus_*Lactococcus*: OR 1.50, 95%CI 1.02-2.20; genus_*Ruminococcus gauvreauii*: OR 2.85, 95%CI 1.75-5.18; genus_Desulfovibrionales: OR 2.06, 95% CI 1.02-4.16) and three gut taxa negatively associated with BM risk (genus_*Eubacterium ventriosum*: OR 0.46, 95%CI 0.25-0.85; genus_*Erysipelatoclostridium*: OR 0.48, 95%CI 0.31-0.76; order_NB1n: ORn 0.56, 95%CI 0.39-0.80). Other algorithms had similar results (Table 1).

Table 1: Association of genetically predicted the causal effect between the gut microbiota and ABI and BM by four different MR methods: IVW, MR Egger, weighted median, weighted mode.

Exposure	No of SNP	Method	OR (95% CI)	P
ABI		NA	-	-
Family_*Lachnospiraceae*	8	IVW	5.14 (1.13-23.33)	0.034
		MR Egger	71 82 (0.00-1728667.13)	0.438
		Weighted median	3.00 (0.40-22.71)	0.287
		Weighted mode	2.08 (0.09-46.84)	0.658
genus_*Butyrieicoceus*	5	IVW	6.53 (1.47-29.02)	0 014
		MR Egger	3 62 (0.16-83.71)	0.481
		Weighted median	4.28 (0.58-31.47)	0.153
		Weighted mode	348 (0.38-32.18)	0.333
BM		NA	-	-
genus_*Eubactetitun ventriosun* group	15	IVW	0.47 (0.26-0.85)	0.013
		MR Egger	0.17 (0.01-2.64)	0.228
		Weighted medion	0.37 (0.17-0.79)	0.010
		Weighted mode	0.33 (0.09-1.20)	0.115
genus_*Lactococcus*	9	IVW	1.50 (1.03-2.21)	0.037
		MR Egger	0.90 (0.16-5.04)	0.910
		Weighted medion	1.37 (0.83-2.26)	0.220
		Weighted mode	1.30 (0.64-2.63)	0.489
geuus_*Rwuinococcus gauvreauii* group	11	IVW	2.86 (1.58-5.18)	0.001
		MR Egger	1.79 (0.16-20.59)	0.652
		Weighted medion	3.09 (1.40-6.82)	0.005
		Weighted mode	3.67 (1.01-13.26)	0.076
genus_*Erysipelatoclostridiwn*	15	IVW	0.49 (0.31-0.76)	0.001
		MR Egger	0.82 (0.14-4.62)	0.821
		Weighted medion	0 4 L (0.23-0.14)	0.003
		Weighted mode	0.40 (0.15-1.06)	0.088
order_Desulfovibrionales	10	IVW	2.07 (1.03-4.16)	0.0.12
		MR Egger	2 44 (008-77.26)	0 626
		Weighted medion	13L (0.52-3.33)	0.567
		Weighted mode	1.14 (0.29-4.52)	0.854
order NB1n	12	IVW	0.56 (0.39-0.80)	0.002
		MR Egger	0.41 (0.10-1.75)	0.256
		Weighted medion	0.59 (0.37-0 9)	0.027
		Weighted mode	0 68 (0.33-1.37)	0.303

Note: SNPs: Single Nucleotide Polymorphisms; ABI: Anoxic Brain Injury; BM: bacterial meningitis; IVW: Inverse Variance Weighted; OR: Odds Ratio; CI: Condential Interval.

Table 2: Characteristics of the study used for primary MR analysis.

Traits	Consortium	Sample size	Cases	Controls	SNPs	Population
Gut microbiota	MiBioGen	18,340	-	-	2037	European, American Hispanic, Eata Asia, etc
ABI	FinnGen	2,05,990	191	2,05,799	16,380,425	European
BM	FinnGen	2,18,059	574	217,485	16,380,461	European

No heterogeneity effect found by Cochran's Q and the P>0.05 in MR-Egger interprets, showing the absence of horizontal pleiotropy (Table 3). Firstly, we visually examined forest plot and funnel plot. Leave-one-out analysis also revealed the robustness of our main results. Finally, four methods were employed to assess the results of MR analysis and the scatter plot was generated for BM and ABI.

Genetic instruments for ABI and BM

Additional information on SNPs, such as effector alleles, beta, se and P-values, was similarly collected when ABI and BM were used as exposure factors.

We found that increased abundance of the Veillonellaceae family, *Lachnospiraceae* NC2004 group and *Eisenbergiella* genus was positivity associated with ABI risk, while the decreased abundance of the *Oscillibacter* genus negative associate with ABI risk, which suggest that *Oscillibacter* genus act as a protective infector in ABI. The risk of BM was positivity associated with the increased abundance of Clostridiales vadin BB60 group family, while the decreased abundance of *Eubacterium hallii* group genus, *Eubacterium ventriosum* group genus and *Erysipelatoclostridium* genus was negativity associated with BM risk. Other algorithms yielded similar results, while significant differences were only observed in IVW (Table 4). No heterogeneity was found by Cochran's Q (P>0.05). We examined forest plot and funnel plot. Leave-one-out analysis also revealed the robustness of our main results. Finally, four methods were employed to assess the results of MR analysis, and the scatter plot was generated for gut flora.

Table 3: MR results of causal links between gut microbiota and ABI and BM.

Eposure	Outcome	Methods	Q-statistic	P-val (Q)	Egger_intercept	P-val (intercept)
family_*Lachnospiraceae*	ABI	MR Egger	4.81	0.56	-0.14	0.62
		IVW	5.08	0.64	-	-
genus_*Butyricicoccus*	ABI	MR Egger	2.52	0.47	0.06	0.7
		IVW	2.7	0.6	-	-
genus_*Eubacterium ventriosum* group	BM	MR Egger	15.59	0.27	0.07	0.47
		IVW	16.24	0.29		
genus_*Lactococcus*	BM	MR Egger	4.53	0.71	0.06	0.56
		IVW	4.89	0.76	-	-
genus_*Ruminococcus gauvreauii* group	BM	MR Egger	5.74	0.76	-0.1	0.72
		IVW	5.89	0.82	-	-
genus_*Erysipelatoclostridium*	BM	MR Egger	4.23	0.98	-0.04	0.55
		IVW	4.59	0.99	-	-
order_Desulfovibrionales	BM	MR Egger	4.34	0.82	-0.01	0.92
		IVW	4.35	0.88	-	-
order NB1n	BM	MR Egger	3.97	0.94	0.03	0.67
		IVW	4.16	0.96	-	-

Table 4: Association of genetically predicted the causal effect between ABI, BM and the gut microbiota by four different MR methods: IVW, MR Egger, weighted median and weighted mode.

Exposure	Outcome	No. of SNP	Method	OR(95% CI)	P
ABI	family_Veillonellaceae	7	IVW	1.02 (1.00-1.04)	0.035
			MR Egger	1.01 (0.86-1.20)	0.89
			Weighted median	1.01 (0 99-1.04)	0.309
			Weighted mode	1.01 (0.97-1.05)	0.693
ABI	genus_*Eisenbergiella*	7	IVW	1.04 (1.00-1.08)	0.035
			MR Egger	1.24 (0.90-1.69)	0.245
			Weighted median	1.02 (0.98-1.06)	0.36
			Weighted mode	1.02 (0.96-1.07)	0.59
ABI	genus_*Lachnospiraceae* NC2004 group	7	IVW	1.04 (1.01-1.07)	0.015
			MR Egger	1.11 (0.85-1.45)	0.489
			Weighted median	1.03(0.99-1.07)	0.113
			Weighted mode	1.03(0.98-1.08)	0.339

ABI	genus_*Oscillibacter*	7	IVW	0.97 (0.95-0.99)	0.018
			MR Egger	0.96 (0.77-1.20)	0.719
			Weighted median	0.97 (0.94-1.00)	0.075
			Weighted mode	0.98 (0.94-1.03)	0.489
BM	family_Clostridiales vadin BB60 group	6	IVW	1.04 (1.00-1.09)	0.037
			MR Egger	1.03 (0.79-1.34)	0.848
			Weighted median	1.04 (0.99-1.10)	0.114
			Weighted mode	1.05 (0.98-1.12)	0.215
BM	genus_*Eubacterium hallii* group	6	IVW	0.97 (0.94-1.00)	0.038
			MR Egger	0.96 (0.77-1.19)	0.715
			Weighted median	0.96 (0.92-1.01)	0.127
			Weighted mode	0.96 (0.90-1.02)	0.267
BM	genus_*Eubacterium ventriosum* group	6	IVW	0.96 (0.93-1.00)	0.036
			MR Egger	0.90 (0.73-1.12)	0.405
			Weighted median	0.96(0.92-100)	0.062
			Weighted mode	0.96 (0 .91-1.01)	0.193
BM	genus_ *Erysipelatoclostridium*	6	IVW	0.95 (0.92-I.00)	0.029
			MR Egger	0.84 (0.64-I.09)	0.265
			Weighted median	0.96 (0.91-1.01)	0.081
			Weighted mode	0.96 (0.90-1.03)	0.345

Note: SNPs: Single Nucleotide Polymorphisms; ABI: Anoxic Brain Injury; BM: bacterial meningitis; IVW: Inverse Variance Weighted; OR: Odds Ratio; CI: Condential Interval.

DISCUSSION

Recently the gut microbiome is recognized as a key regulator of host healthy. The gut microbiota effect on the host may through the metabolome, transcriptome, and epigenome pathways [22,23]. With the unveiling of the "gut-brain axis", it has been found that the connection can be through: (1) Bacterial components such as lipopolysaccharides stimulate the immune system to produce systemic or CNS inflammation [24]; (2) Bacterial proteins cross over with antigens to stimulate dysfunction in adaptive immunity [24]; (3) Bacterial enzymes produce neurotoxins and neurological metabolites[25]; (4) Gut microbiota produce hormones and neurotransmitters [26,27]; (6) Intestinal bacteria directly stimulate adaptive immunity [28].

In this bidirectional MR study, we found a causal relationship between gut microbiota and ABI and BM. The *Lachnospiraceae* families and *Butyricicoccus* genus are all Firmicutes and positively associated with the risk of ABI. In a reverse causality test, ABI altered the gut microbiota, with increased abundance of the *Lachnospiraceae* families. *Butyricoccus spp*. is related to butyrate production a Short-Chain Fatty Acids (SCFA), so we speculate that butyrate may act as a risk factor in ABI. Animal studies have found that *Butyricoccus* genus was increased significantly in the infected mice that may associated with an upregulation of inflammation response in the intestines [29]. However, the results of this MR analysis are contrary to other brain-gut axis diseases [5,30]. Previous studies have demonstrated the butyrate can promote the process of renewal and repair to intestinal cell, as well as enhance the function of immune cell. However, there are little clinical studies about the state of the intestines in patients with ABI. It's still not clear whether this paralleled level of *Butyricoccus spp*. to intestinal inflammation exists in ABI patients. Only it can be confirmed by numerous of subsequent clinical and animal experiments. Reversely, the decreased abundance of *Lachnospiraceae* family is negatively associated with severity in other CNS diseases, such as depression and Parkinson [30,31]. Meanwhile, previous studies of hypoxic or ischemic-hypoxic brain injury have shown a decrease in the proportion of Firmicutes [5]. It's difficult to conclude they are protective or risk factor, because of *Lachnospiraceae* family and *Butyricoccus* genus as a member of the Firmicutes. Patnala' team shown that butyrate regulates H3K9ac and enhances neuroprotection of microglia cell at the gene level during stroke [32]. However, this MR research did not involve fecal metabolites, so it is difficult to determine the role of butyrate in ABI, which needs to be confirmed by further animal and clinical studies.

The increased abundance of *Lactococcus* genus is positively associated with the risk of BM. Since the 1990s, there have been successive case reports of blood-borne infections caused by the *Lactococcus* genus [33,34]. The *Ruminococcus gauvreauii* genus is a bacterium isolated from human bile. Djawad, et al. found that *Ruminococcus gauvreauii* genus is involved in the synthesis of glutamate, butyric acid, 5-hydroxytryptamine, all these neurotransmitters are associated with depression [35]. The Desulfovibrionales genus, one of the *Aspergillus*, can use lactic acid, pyruvic acid and ethanol as carbon sources to reduce sulphate to hydrogen sulphide, the latter of which is closely associated with inflammatory responses in the host and as a risk factor for inflammation. Clinical studies have shown that the abundance of Desulfovibrionales genus correlates with the severity of Parkinson's and the mechanism may be related to the fact that Desulfovibrionales genus can produce hydrogen sulphide and lipopolysaccharide or induce oligomerisation of a-synuclein [36,37].

CONCLUSION

In summary, defining the characteristics of a bacterium requires placing it in the context of the entire microecosystem and discerning its abundance threshold and functional characteristics through the composition and metabolism of the entire ecological flora. Although the abundance of some of the bacteria in our study served as a disease risk factor, which contradicts of other studies, we believe that the gut flora acts as a dynamic equilibrium to influence the host and the pattern of response varies with different diseases. It's still difficult to define clearly link between the gut microbiota and the host by the results of the current clinical and animal studies.

Despite the rigorous statistical methods used in this study, limitations still exist in this study. Although we used linear MR analyses, the lack of specific sample information, it was not possible to conduct further observational analyses on the age and sex of the exposure and outcome populations. Furthermore, our IVs were screened for $P<1 \times 10^5$, and the results may have been affected by weak instrumental bias.

Using a two-sample bidirectional MR analysis, our study confirmed a bidirectional causal relationship between gut microbial abundance and the risk of ABI and BM. Among this, a strong association between elevated abundance of *Lachnospiraceae* family and the risk of ABI, which may serve as a potential target for the treatment of ABI?

CONSENT FOR PUBLICATION

Informed consent was obtained from all subjects involved in the study.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on request from corresponding author.

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AUTHOR'S CONTRIBUTIONS

Conceptualization, Jia An, Mingtang Ye; data collection, Jia An; data analysis and interpretation, Di Yu, Qingfeng Wang; software and statistical analysis, Qiang Wang, Kede Wu; writing-original draft preparation, Jia An, Zhaocong Yang, Xuming Mo. All authors contributed to the final version of the manuscript and agreed to the published version of the manuscript.

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