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The causal effect of gut microbiota on hepatic encephalopathy: a mendelian randomization analysis

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Abstract

Background There is growing evidence for a relationship between gut microbiota and hepatic encephalopathy (HE). However, the causal nature of the relationship between gut microbiota and HE has not been thoroughly investigated.

Method This study utilized the large-scale genome-wide association studies (GWAS) summary statistics to evaluate the causal association between gut microbiota and HE risk. Specifically, two-sample Mendelian randomization (MR) approach was used to identify the causal microbial taxa for HE. The inverse variance weighted (IVW) method was used as the primary MR analysis. Sensitive analyses were performed to validate the robustness of the results.

Results The IVW method revealed that the genus Bifidobacterium (OR = 0.363, 95% CI: 0.139-0.943, $P = 0.037$), the family Bifidobacteriaceae (OR = 0.359, 95% CI: 0.133-0.950, $P = 0.039$), and the order Bifidobacteriales (OR = 0.359, 95% CI: 0.133-0.950, $P = 0.039$) were negatively associated with HE. However, no causal relationship was observed among them after the Bonferroni correction test. Neither heterogeneity nor horizontal pleiotropy was found in the sensitivity analysis.

Conclusion Our MR study demonstrated a potential causal association between Bifidobacterium, Bifidobacteriaceae, and Bifidobacteriales and HE. This finding may provide new therapeutic targets for patients at risk of HE in the future.

Keywords Mendelian randomization, Gut microbiota, Hepatic encephalopathy, Causal relationship, Gut-liver-brain axis

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Introduction

Hepatic encephalopathy (HE) is a reversible neurocognitive impairment that commonly occurs in patients with liver cirrhosis and other advanced liver diseases [1]. HE, with its broad range of neurological symptoms from mild cognitive changes to severe confusion and coma, significantly impairs patients' quality of life and contributes substantially to increased hospitalizations, morbidity rates, and mortality risks [2, 3]. The pathogenesis of HE is complex and multifactorial, involving liver failure, hyperammonemia, systemic inflammation, and infection [4, 5]. In recent years, a mounting body of research findings has supported alterations of the gut microbiota play

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an important role in HE pathogenesis [6–9]. In particular, the perturbation of the crosstalk among gut, liver, and brain, often referred to as the "gut-liver-brain" axis, has been suggested to be a determinant factor in the development and severity of HE in patients with liver cirrhosis. A previous study has indicated that elderly patients, aged over 65 years, with cirrhosis tend to exhibit an altered gut–liver–brain axis, characterized by impaired cognitive performance, particularly affecting memory, in comparison to age-matched noncirrhotic controls [10]. In a randomized clinical trial (RCT), Bajaj et al. found that fecal microbiota transplantation from a rationally selected donor for cirrhotic patients with recurrent HE was safe, and associated with lower hospitalizations, improved cognition compared with whose received standard of care treatment [8]. Although changes in the gut microbiota composition and function have critical relationship with liver health and brain function, previous observation studies may encounter several obstacles in establishing a causal relationship between gut microbiota and HE due to limitations in sample size, population heterogeneity, and the presence of potential confounding factors.

Alternative methodologies aimed at reinforcing the causal association between the gut microbiota and HE will serve to determine the potential necessity of conducting RCTs and offer valuable insights for the advancement of targeted therapeutic interventions. Mendelian randomization (MR) offers an opportunity to infer the potential causal relationship between an exposure and an outcome through the utilization of genetic variants as instrumental variables (IVs) [11]. This method can help to address confounding and minimize the possibility of reverse causality, as genetic variants that are randomly assorted at conception and not influenced by the development or progression of the disease. Herein, we conducted a two-sample MR analysis of gut microbiota in relation to the risk of HE using the large genome-wide association studies (GWAS).

Methods

Study design and data sources

To investigate the potential causal relationship of the gut microbiome with the risk of HE, we applied a two-sample MR analysis. An overview of the study design is illustrated in Fig. 1.

Genetic variants for gut microbiota were obtained from the largest GWAS conducted by the MiBioGen consortium [12]. This study including 18,340 individuals of European ancestry from 24 population-based cohorts. After eliminating fifteen previously unidentified gut microbiome taxa, a total of 196 known gut microbiome taxa were retained for further analysis. The GWAS summary statistics for HE was obtained from FinnGen consortium R8 release data [13]. This GWAS study utilized the phenotype of "Encephalopathy" and encompassed a cohort consisting of 225 cases and 318,912 controls. The study followed the STROBE reporting guideline [14], and ethical approval was not required since all data were obtained from publicly accessible summary statistics.
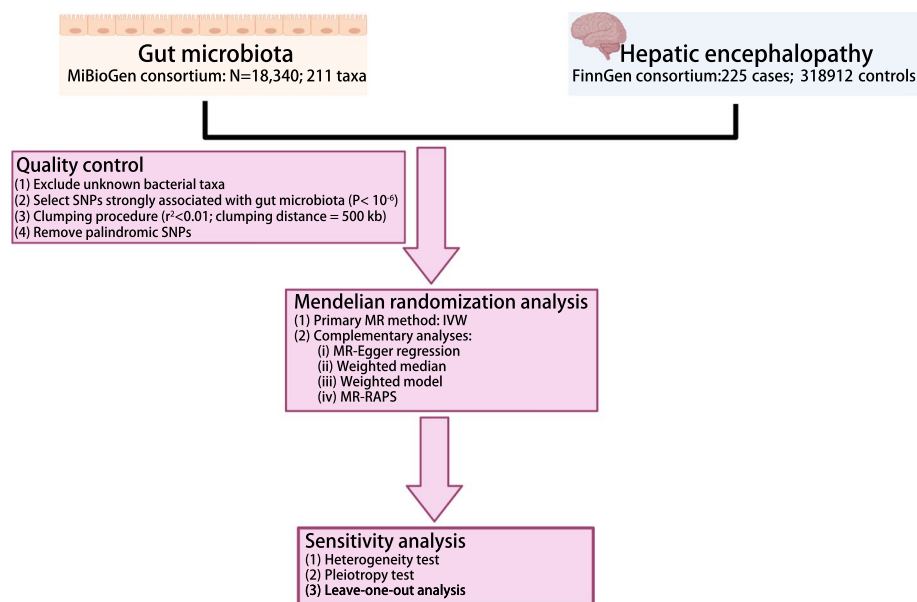
Fig. 1 Flow chart of the present MR study showing the causal association between gut microbiota and hepatic encephalopathy. SNPs, single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance-weighted; MR-RAPS, MR-Robust adjusted profile score

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Genetic instrument selection

To obtain reliable results, the following assumptions must be satisfied [15]: (1) single nucleotide polymorphisms (SNPs) strongly associated with each gut microbiome taxa were selected as IVs; (2) all IVs are not associated with any potential confounding factors and (3) the IVs solely influence the HE through the gut microbiota and not through any alternative pathways. Due to the smaller IVs obtained by the genome-wide significance threshold ($P < 5\times 10^{-8}$), a more relaxed threshold ($P < 1.0\times10^{-6}$) was used. To mitigate potential bias resulting from allelic association, the linkage disequilibrium was set to $r^2 < 0.01$ with clumping distance of 500kb.

Statistical analysis

The inverse variance weighted (IVW) method was used as the primary statistical method in this study. Additionally, complementary analyses were conducted using MR-Egger regression, weighted model, weighted median, MR-robust adjusted profile score (MR-RAPS) as alternative methods. In order to evaluate the robustness of significant findings, a series of sensitivity analyses were conducted. Cochrane's IVW Q test was performed to test the heterogeneity of IVs. The assessment of potential pleiotropy was initially determined by the intercept of MR Egger intercept test. Scatter plots were employed to visualize the results. Furthermore, leave-one-out plots were built to evaluate the relationship between each SNP within gut microbiome taxa and HE risk.

The F-statistic was used to assessed the strength of IVs using the formula $F = \frac{R^2(n-K-1)}{K(1-R^2)}$ (R^2: proportion of variance in the gut microbiota explained by SNPs; n: sample size; K: the number of IVs). F statistic greater than 10 was considered n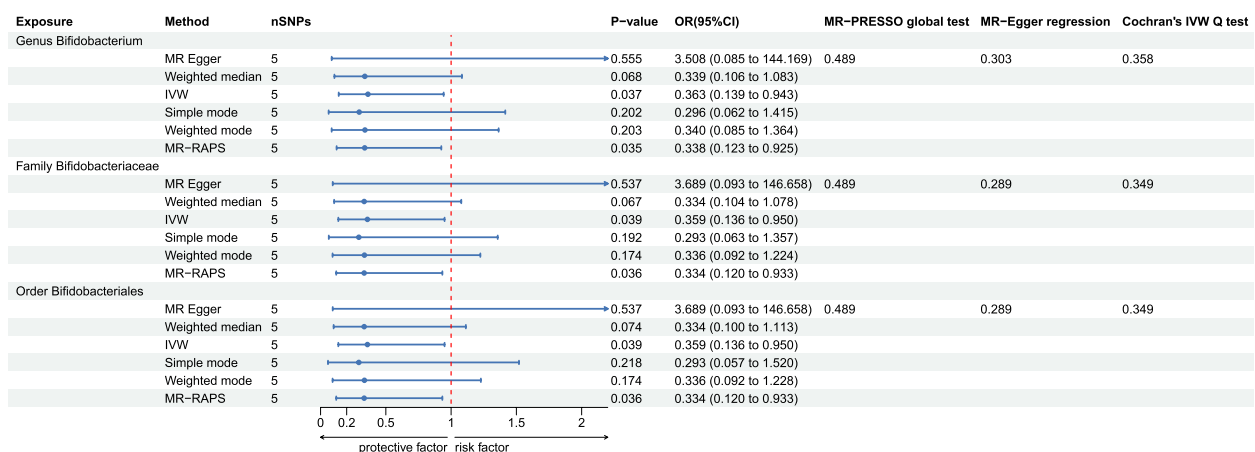o significant weak IVs bias. We conducted Bonferroni correction test for multiple testing, and an adjusted P value ($P < 0.05/N$, N represents the effective number of bacterial taxa at each feature level).

All data cleaning and statistical analyses were performed by using packages "TwoSampleMR" (version 0.5.6), "MRPRESSO" (version 1.0) in R software (Version 4.2.3, https://www.r-project.org/). The odds ratio (OR) was utilized to reveal the influence of gut microbiota on the risk of HE. $P < 0.05$ was deemed indicative of evidence for a potential causal effect.

Results

According to the selection criteria of IVs and a series quality control step, two hundred and ninety independent SNPs associated with 196 bacterial taxa were selected as IVs, including 30, 59, 149, 32, and 20 SNPs at the class, family, genus, order, and phylum levels, respectively. Details about the selected instrumental variables are shown in Table S1. After removing SNPs with palindromic sequences and intermediate allele frequencies, we finally obtained 94 SNPs as IVs to assess the causal association of gut microbiota with HE in this study. All these IVs had F statistics greater than 20 (range 20.631-88.430), suggesting that there was no weak IVs for the results (Table S2).

As shown in Fig. 2, three bacterial genera, specifically, the genus Bifidobacterium, the family Bifidobacteriaceae, and the order Bifidobacteriales, were found to associated with HE in at least one MR method. The IVW method indicates that the genus Bifidobacterium had a protective effect on HE (OR = 0.363, 95% CI: 0.139-0.943, P = 0.037). The IVW estimate of the family Bifidobacteriaceae also showed its suggestive protective effect against HE (OR = 0.359, 95% CI: 0.133-0.950, P = 0.039).

Exposure	Method	nSNPs		P−value	OR(95%CI)	MR−PRESSO global test	MR−Egger regression	Cochran's IVW Q test
Genus Bifidobacterium								
	MR Egger	5		0.555	3.508 (0.085 to 144.169)	0.489	0.303	0.358
	Weighted median	5		0.068	0.339 (0.106 to 1.083)			
	IVW	5		0.037	0.363 (0.139 to 0.943)			
	Simple mode	5		0.202	0.296 (0.062 to 1.415)			
	Weighted mode	5		0.203	0.340 (0.085 to 1.364)			
	MR−RAPS	5		0.035	0.338 (0.123 to 0.925)			
Family Bifidobacteriaceae								
	MR Egger	5		0.537	3.689 (0.093 to 146.658)	0.489	0.289	0.349
	Weighted median	5		0.067	0.334 (0.104 to 1.078)			
	IVW	5		0.039	0.359 (0.136 to 0.950)			
	Simple mode	5		0.192	0.293 (0.063 to 1.357)			
	Weighted mode	5		0.174	0.336 (0.092 to 1.224)			
	MR−RAPS	5		0.036	0.334 (0.120 to 0.933)			
Order Bifidobacteriales								
	MR Egger	5		0.537	3.689 (0.093 to 146.658)	0.489	0.289	0.349
	Weighted median	5		0.074	0.334 (0.100 to 1.113)			
	IVW	5		0.039	0.359 (0.136 to 0.950)			
	Simple mode	5		0.218	0.293 (0.057 to 1.520)			
	Weighted mode	5		0.174	0.336 (0.092 to 1.228)			
	MR−RAPS	5		0.036	0.334 (0.120 to 0.933)			

0 0.2 0.5 1 1.5 2
protective factor risk factor

Fig. 2 The MR results for association between gut microbiota and HE. IVW, inverse variance-weighted; MR-RAPS, MR-Robust adjusted profile score. HE, hepatic encephalopathy

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Similarly, the IVW estimate suggests a potential protective effect of the order Bifidobacteriales on HE (OR = 0.359, 95% CI: 0.133-0.950, *P* = 0.039). The MR-RAPS method also consistently shows that the genus Bifidobacterium (OR = 0.338, 95% CI: 0.123-0.925, *P* = 0.035), the family Bifidobacteriaceae (OR = 0.334, 95% CI: 0.120-0.933, *P* = 0.0363), and the order Bifidobacteriales (OR = 0.334, 95% CI: 0.120-0.933, *P* = 0.0363) are associated with a reduced the risk of HE. However, after multiple-testing correction, no causal effect of the three identified taxa on HE was found using an adjust *P* value.

The scatter plot also shown the protective effect of the three bacterial taxa on HE (Figure S1 A-C). Although rs7322849 of Bifidobacterium, Bifidobacteriaceae, and Bifidobacteriales appeared as potential outliers in scatter plots and leave-one-out plots (Figure S1 D-F), additional MR-PRESSO analysis did not identify any significant outliers with a global test *P* value greater than 0.05. Consequently, there was inadequate evidence to suggest horizontal pleiotropy in the relationship between these bacteria and HE. Meanwhile, the result of leave-one-out analysis revealed no single SNP is driving the causal associations between the identified bacterial taxa and HE, indicating our results were stability. For all the 3 bacterial taxa mentioned above, no heterogeneity and pleiotropy were found (Fig. 2, Table S3-4).

Discussion

In the current study, we performed a two sample MR analysis to investigate the causal relationship between gut microbiome and HE. We identified several genera of gut microbiota, including the genus Bifidobacterium, the family Bifidobacteriaceae, and the order Bifidobacteriales, which exhibited a suggestive potential protective causal effect on the risk of HE.

The gut milieu plays a pivotal role in the pathogenesis of complications associated with cirrhosis. Dysbiosis within the gut microbiome can initiate a cascade of inflammatory responses and subsequent activation of immune cells, ultimately promoting hepatic fibrosis and disease progression [16]. HE is a common and severe complication that arises from both chronic liver disease and acute liver failure, leading to notable impairment in cerebral function. The relationship between gut microbiota and HE has established in previous observational studies [6, 8, 17]. Malaguarnera et al. observed that the administration of a combination of Bifidobacterium plus fructo-oligosaccharides for a duration of 60 days resulted in a significant improvement in psychometric tests and blood ammonia levels compared to the lactulose group in patients with HE [18]. The MR analysis results also revealed that the genus Bifidobacterium reduce the risk of HE in this study. Consistent with previous studies [19,

20], we also found that the family Bifidobacteriaceae and the order Bifidobacteriales were protective effect against HE. In a Phase 1 RCT, Bajaj and colleagues discovered that the administration of oral fecal microbial transplant (FMT) capsules was linked to notable improvements in duodenal mucosal diversity, dysbiosis, antimicrobial peptides expression, and improved cognition among patients with cirrhosis and recurrent HE [19]. Outcomes from this study also revealed increased abundances of Ruminococcaceae and Bifidobacteriaceae in FMT group, while decreasing the abundances of Streptococcaceae and Veillonellaceae. These findings support the potential of FMT in modulating the gut microbiota and improving the inflammatory profile in patients with hepatic encephalopathy by reducing the expression of IL-6 and simultaneously increasing the expression of key antimicrobial peptides as well as intestinal barrier proteins [21, 22].

The gut-liver-brain axis is a complex network of interactions between the gut microbiota, liver, and brain which plays a crucial role in various physiological and pathological processes. This relationship is influenced by various factors such as diet, individual behaviors, environment factors, and surgical procedures like the transjugular intrahepatic portosystemic shunt [23, 24]. Besides, alterations in the gut ecology may induce gut dysbiosis and develop of series complications. HE is recognized as a representative model of a disease within the gut-liver-brain axis (Fig. 3), despite its pathogenesis remaining inadequately comprehended. Recently, increasing data demonstrates the alterations observed in the composition and function of gut microbiota have been linked to an increase in intestinal permeability, thereby facilitating the translocation of bacterial products, notably endotoxins, into the systemic circulation [25–27]. These microbial products can then activate immune and inflammatory responses in the liver and brain, contributing to the development and progression of HE. Furthermore, alterations in gut microbial taxa can serve as a vulnerability factor, influencing the neuroendocrine and neuroimmune signaling pathways within the gut-brain axis, thereby potentially impacting neurological function and behaviors [28, 29]. Moreover, an increasing body of research has demonstrated a link between liver fibrosis and inferior cognitive performance, with serum C-reactive protein playing a significant mediating role, suggesting the potential involvement of systemic inflammation in the liver-brain axis [30, 31]. These findings provide compelling evidence for the critical role played by gut microbiota in the development and progression of HE. However, most of the previous studies on the relationship between intestinal flora and HE were predominance of observational studies and mouse models. It's challenging to provide robust evidence regarding the

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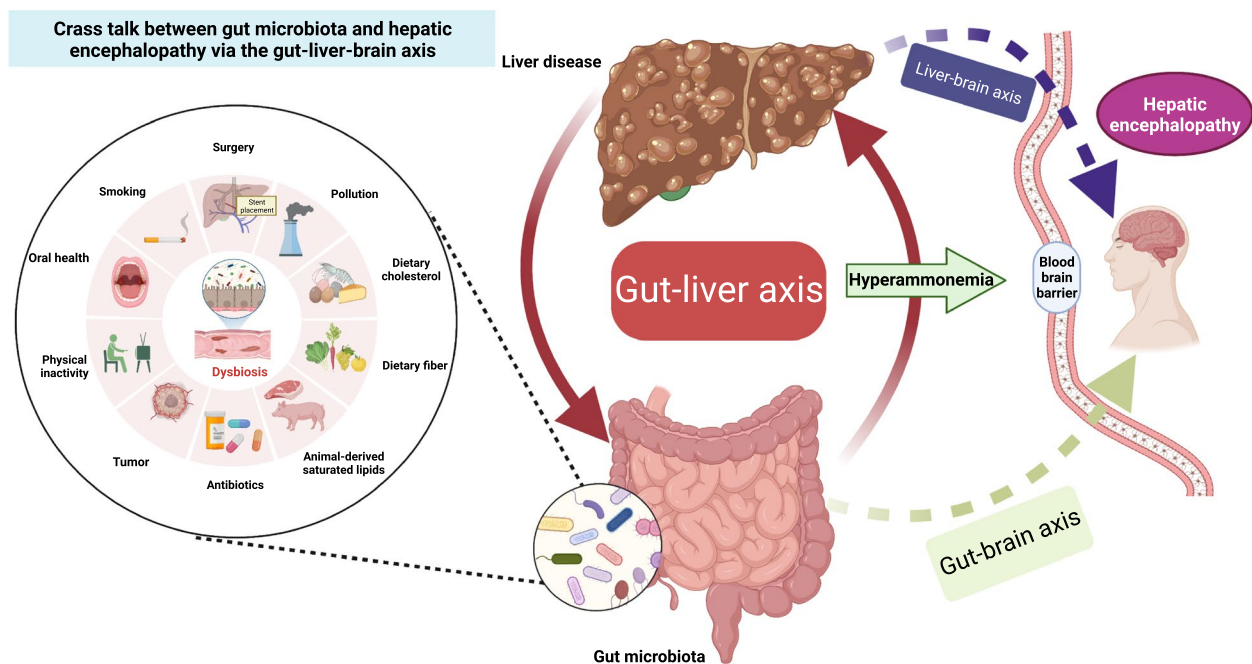
Fig. 3 Crass-talk between gut microbiota and hepatic encephalopathy via the gut-liver-brain axis

causal association between the gut microbiota and HE due to confounding factors.

By utilizing genetic variants as IVs, MR analysis mimics the randomization in clinical trials and helps overcome potential confounding factors, thus, minimizing bias in causal effect estimates. Using two-sample MR analysis, we investigated the causal relationship between the gut microbiota and HE. To ensure the robustness of instrumental variables in the MR analysis of gut microbiota, genetic variants were obtained from the largest available GWAS meta-analysis [12]. In line with prior researches [19, 32], both the IVW and MR-RAPS method results indicated increasing abundant levels of the genus Bifidobacterium, the family Bifidobacteriaceae, and the order Bifidobacteriales are genetically associated with decreased HE risk. The IVW method assumes the validity of all instrumental variants based on MR assumptions and combines the Wald ratios for each SNP to yield a pooled estimate [33]. It is considered the most potent method for MR estimation, but estimates derived from this approach may be susceptible to bias in the presence of directional pleiotropy. MR-RAPS, an extension of the IVW method, serves as a correcting model in the presence of pleiotropy. It offers enhanced robustness against deviations from the underlying assumptions of MR due to its ability to adjust the profile likelihood of the summary data employed in the analysis [34]. While the multiple test results suggest the possibility of false positives and

reveal no significant genetic correlation between the microbiota and HE risk, we believe that this may be partly due to the conservative nature of the Bonferroni correction and the relatively small sample size [35, 36].

There were several limitations in our study. First, due to using summary data rather than raw data, it is difficult to explore the stratification effects and perform subgroup analysis, such as distinguishing alteration of gut abundance in chronic liver disease and acute liver failure, and type and clinical grading of HE. Besides, since this study specifically examines the effects within a European descent population, caution should be exercised when generalizing the results to other ethnicities without additional justification. Therefore, it is crucial to conduct further RCTs with clear classification and inclusion of diverse ethnic groups to establish a comprehensive understanding of the relationship between gut microbiota and HE. Second, in order to perform sensitivity analysis and detect potential horizontal pleiotropy, SNPs used in this two-sample MR analysis may not have reached the conventional GWAS significance threshold of $P < 5 \times 10^{-8}$. For this, the F-statistic was used to assessed the strength of IVs and Bonferroni correction test was performed to mitigate the risk of false positives. Third, the definition of "hepatic encephalopathy" has varied across previous studies [2, 37], leading to complexities in its classification. While our findings align with previous studies, it is important to note that the causes of HE among the participants included in this study may be diverse.

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In conclusion, we revealed evidence of three bacterial taxa, specifically, the genus Bifidobacterium, the family Bifidobacteriaceae, and the order Bifidobacteriales, may have a protective effect against HE. Our study findings shed light on the complex cross-talk between the gut microbiota and HE through the gut-liver-brain axis, which may provide a novel way for the development of targeted therapies. In the future, further large population-based longitudinal research and more diverse cohorts are required to verify our findings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-024-01939-y.

> Supplementary Material 1.
>
> Supplementary Material 2.

Availability of data and materials
All data generated during this study are provided in the original publications and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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