Gut-brain Axis: Relationship Between the Gut Microbiome and Autism Spectrum Disorder

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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that impacts children's social interactions. Although the mechanisms are not well understood and treatments are lacking, recent studies on the brain-gut axis offer insights into ASD pathology. Differences in gut microbiota between children with ASD and healthy controls have been reported, with my study identifying a significant increase in Enterobacteriaceae in ASD children. This finding aids in understanding ASD's pathogenesis via the gut-brain axis, identifying related biomarkers, and developing novel personalized treatments.

***Keywords*:** Autism spectrum disorder, gut-brain axis, microbiota

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by profound impairments of social interactions, highly restricted interests, and repetitive behaviours in early life (Lord et al. 2020). The global prevalence of ASD was estimated at 0.76% by the Global Burden of Disease Study conducted in 2010, but only 16% of the global children population was considered (Baxter et al. 2015). In the United States alone, for 2020, it has been estimated that almost 3% (or 1 in 36) of children aged 8 years had ASD, which is higher than the estimate from previous Autism and Developmental Disabilities Monitoring Network (ADDM) 2000-2018 (Maenner et al. 2023). The need for resources to offer extra medical care for this population of children is also growing with the increasing prevalence, which has been imposing a higher economic burden on families and society. In comparison with a healthy child, a childhood ASD patient is associated with approximately $3,000 higher healthcare costs and around $14,000 higher aggregate non-healthcare costs (Lavelle et al. 2014). Furthermore, the total cost of ASD and associated morbidities was estimated to be $41.8 billion in China in 2020 (Zhao et al. 2024).

Over the past decades, scientists have been trying to elucidate the molecular mechanisms underlying the pathobiology of ASD (Lord et al. 2020). Previous studies have shown that both genetic predispositions and environmental factors such as diet and stress might contribute to the development and progression of ASD (Almandil et al. 2019; Matsuzaki et al. 2012). Recent research has depicted that the gut-brain axis or pathways that connects the gastrointestinal (GI) tract and the central nervous system (CNS) may play a role in the development of a variety of neurological and psychiatric diseases such as Parkinson's disease, Alzheimer's disease, major depressive disorder, and schizophrenia (Kim and Shin 2018). In terms of the association between gut microbiome and ASD, it has been shown that the mothers of children with ASD have altered gut microbiomes and children with ASD also harbour unique bacterial biomarkers (Li et al. 2019). Nevertheless, pinpointing which specific microbiome components play pivotal roles in ASD development remains challenging. Research efforts aimed at elucidating the precise relationship between ASD and specific gut microbiome constituents have yielded conflicting and inconclusive results. While some studies suggest direct links between certain bacteria and ASD, others contest these claims (Finegold et al. 2010; Kang et al. 2013).

In this study, two sets of data involving autistic children were used. Notably, these studies differed in terms of kinship among the participants, with one study comprising siblings (ASD and non-ASD) and the other not. Within the kinship group, no distinction in microbiome richness, structural composition, and core microbiome constituents were found. Interestingly, variations in the Enterobacteriaceae family were consistently observed across both study groups, suggesting a potential direct association with ASD development. This led us to posit that the similarities in diet among kinship group participants may have masked underlying differences. To validate the hypothesis, a Fisher analysis was conducted on the non-kinship group, ensuring that the observed differences were not confounded by age or gender factors. This study a significant step towards unraveling this complex nexus and offer a promising avenue for advancing my understanding of ASD etiology, ultimately paving the way for more effective interventions and treatments.

2. Materials and methods

2.1 Data Sources and Tools

Data were sourced from previous publications (Coretti et al. 2018; Ma et al. 2019). There are total of 371 samples in the two datasets. One of the studies examined the children with ASD and their healthy siblings who are two years apart in age (study ASD_sibling, sample size was 117, of which 60 were patients with ASD), and the other study investigated children with ASD and healthy people (study ASD_health, 254 samples, 111 of which were ASD patients).

The microbiome data were analyzed by Microbiome Analyst 2.0 (Lu et al. 2023). Statistical tests were performed using Python, and figures were prepared using R.

2.2 Data Analysis

For the low count filter, 4 was chosen to be the minimum count, and 10 was used for prevalence in samples (%). The low variance filter is based on the inter-quantile range. All data were neither transformed nor rarefied and total sum scaling (TSS) was used for normalization.

The genus was utilized as taxonomy level, and the top 15 taxa were selected as top n taxa, representing the data with percentage abundance. In terms of the alpha-diversity, the genus was used as the taxonomy level, Shannon Diversity was employed as the measure, and the Mann-Whitney test was selected for examining the statistical significance. To further investigate the structure of the gut microbiome, a beta-analysis was created using the genus as the taxonomy level. The PCoA parameter with PERMANOVA was performed to test statistical significance and the Bray-Curtis Index is the chosen distance method. To precisely find the microbiome that has the largest effect, I used the genus as the taxonomy level, with a sample prevalence cutoff of 20% and a relative abundance of 0.01. While further investigating, I applied a p-value cutoff of 0.1 and used a Log LDA score of 2.0. The LEfSe result table was employed to find the differential bacterium and pick out the bacterium. The Disbiome database (Janssens et al. 2018) was used for inquiring about the detailed association between Enterobacteriaceae and ASD.

3. Results

3.1 Significant differences were observed in microbial communities between age- and sex-matched ASD and healthy children

In study 1, a Fisher analysis was completed, and the number of males and females in the typically developing individuals' group (TD group) and ASD group were analyzed. There were 99 males and 12 females in the ASD group, 130 males and 13 females in the TD group, and the difference was not significant (Fisher test, $p = 0.676$). In addition, the mean age distribution of the TD group is 4.93 with a standard deviation of 1.85 and the mean age distribution of the ASD group is 5.09 with a standard deviation of 1.99. There is no statistical significance in age (Wilcoxon ranksum test, $p = 0.59$). Overall, no significant differences in age and gender were observed between the TD and ASD groups.

The data from study 1 were further analyzed by taxa abundance, alpha-diversity, beta-diversity, and core microbiome composition, to identify significant differences in the distribution of the intestinal microbiota between TD individuals and ASD patients. As shown in Figure 1A and 1B, there were significant differences in the relative abundance of dominant bacteria between the ASD and TD groups. The ASD group has a lower percentage of Bacteroides (ASD: 32.06%, TD: 35.1%), Prevotella (ASD: 2.91%, TD: 0.07%), but has a larger percentage of Dialister (TD: 2.29%, ASD: 4.17%).

To reveal the difference in the diversity of the intestinal microbiome between the TD and ASD groups, the Shannon index which is commonly used to measure the diversity of microbial communities was calculated. The Shannon index of the ASD group (2.801) was significantly higher than that of the TD group (2.702) (Wilcoxon ranksum test, $p = 0.001$) (Figure 1B). At the same time, the results demonstrated the significant difference in the microbial community structure between the ASD and the TD groups (PERMANOVA, $R2 = 0.02$, $p = 0.002$) (Figure 1D).

Then, the core microbiome and the LEfSe analysis were used to examine the different compositions of the microbiome. The analysis revealed that there are significant differences between the top 15 bacteria content, and LEfSe analysis also revealed 170 differential bacteria (Figure 1E). We found that the differential microbiome found by LEfSe analysis is highly similar to the results the core microbiome analysis gave. In the ASD cohort, core microbiomes with positive LDA scores exhibit an increased prevalence compared to TD counterparts. Dialister, for instance, displays a prevalence of 0.5 in TD and a notably higher prevalence of 0.8 in ASD within the 0.01 relative abundance (%). Similarly, the Lachnospiraceae_NK4A136_group presents with a prevalence of 0.8 in TD and a further heightened prevalence of 0.9 in ASD within the 0.01 relative abundance. The core microbiome Subdoligranulum is prevalent at 0.7 in ASD but conspicuously absent in

TD at a 0.01 relative abundance. Escherichia demonstrates a prevalence of 0.2 in TD and an increased prevalence of 0.4 in ASD within the 0.01 relative abundance. Additionally, Eubacterium_coprostanoligenes_group (0.3 in TD, 0.6 in ASD) and Ruminococcaceae_UCG_002 (0.6 in TD, 0.7 in ASD), show increased prevalence in ASD, while Ruminococcus_1 (0.4 in ASD) and Christensenellaceae_R_7_group (0.4 in ASD) exhibit a higher prevalence in ASD but absent in TD group.

Conversely, the TD cohort gave negative LDA scores, and showed distinctive microbial taxa with higher prevalence in this group compared to ASD. Eubacterium_eligens_group (0.8 in TD, 0.6 in ASD), Parabacteroides (0.9 in TD, 0.8 in ASD), and Prevotella_9 (0.8 in TD, 0.4 in ASD), and megamonas (0.4 in TD, 0.1 in ASD) all exhibit increased prevalence in TD compared to ASD within the 0.01 relative abundance

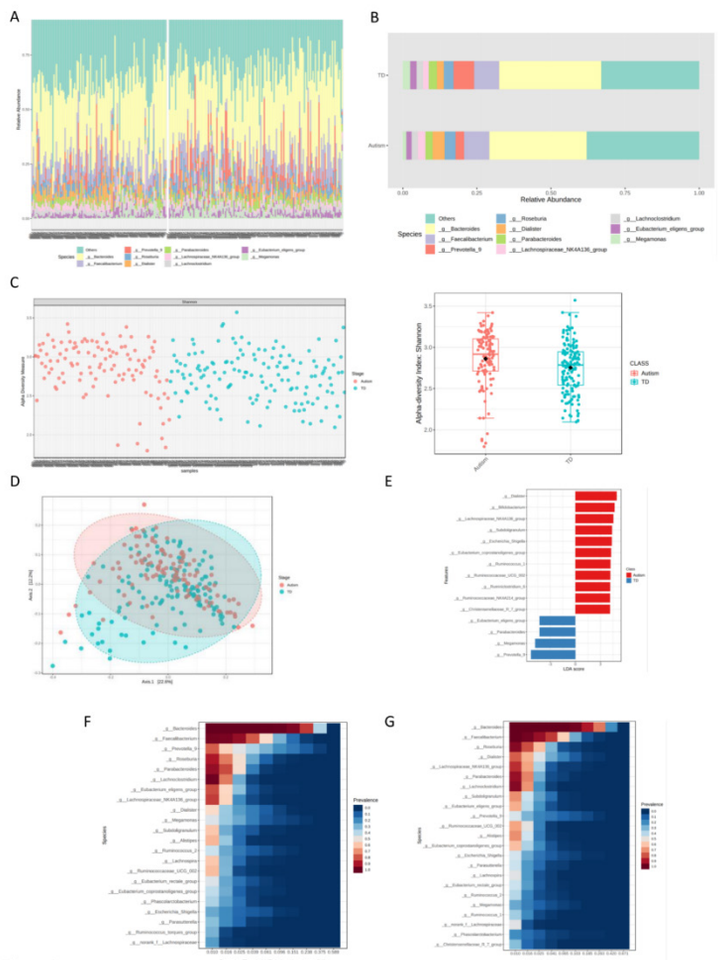

Figure 1.

Figure 1. The microbial communities between age - and sex-matched ASD and healthy children are significantly different. (A) Stacked bar analysis (organized samples). (B) Stacked bar analysis (merged samples). (C) Alpha-diversity. (D) Beta-diversity. (E) LefSe table. (F) Core microbiome analysis (TD group). (G) Core microbiome analysis (ASD group).

3.2 No significant differences are observed in microbial communities between age — and sex-matched ASD and healthy children

Surprisingly, the analysis of data from study 2 produced unexpected results. Unlike study 1, no significant patterns or differences in taxa abundance, microbial community diversity, beta-diversity, or core microbiome composition are observed between ASD patients and their typically developing siblings.

As shown in Figure 2A and 2B, it can be found that the percentage that different intestinal microbiomes occupy has a slight difference between the TD group and the ASD group. The percentage of Bacteroides in TD is 35.8% compared to that of ASD is 34.8%. To detect the particular level of the difference, a microbial community diversity graph was carried out (Figure 2D). The microbial community diversity of children with ASD was 2.55, and the TD group was 2.52 (Shannon index, $p = 0.30144$). Then, the Beta-diversity analysis indicated that the microbial community changed significantly (PERMANOVA, $R2 = 0.00896$, $p = 0.385$). the core microbiome and the LEfSe analysis were employed to find the core microbiome in TD

and ASD (Figure 2E). The results reveal that there are no significant differences between the top 15 bacteria content, and LEfSe analysis also revealed 12 differential bacteria.

3.3 No specific pattern was found by LEfSe analysis

The LEfSe analysis failed to reveal specific patterns in the results. However, a noteworthy discovery emerged from the differential bacterium identified in the LEfSe analysis - the Enterobacteriaceae family. This bacterium was found to be present in both studies (Figure 2 F, G, and H), occupying a certain proportion, and has been linked to many mental health conditions. The repeated identification of this differential bacterium across multiple studies may suggest its potential association with ASD and other mental diseases.

To shed further light on the Enterobacteriaceae family, additional analyses were conducted on the Disbiome databases. The findings provided insights into the potential role of this bacterium in mental health, reinforcing the notion that it may be associated with ASD. Enterobacteriaceae is known to have a relationship with ASD which has been previously reported in the Disbiome database.

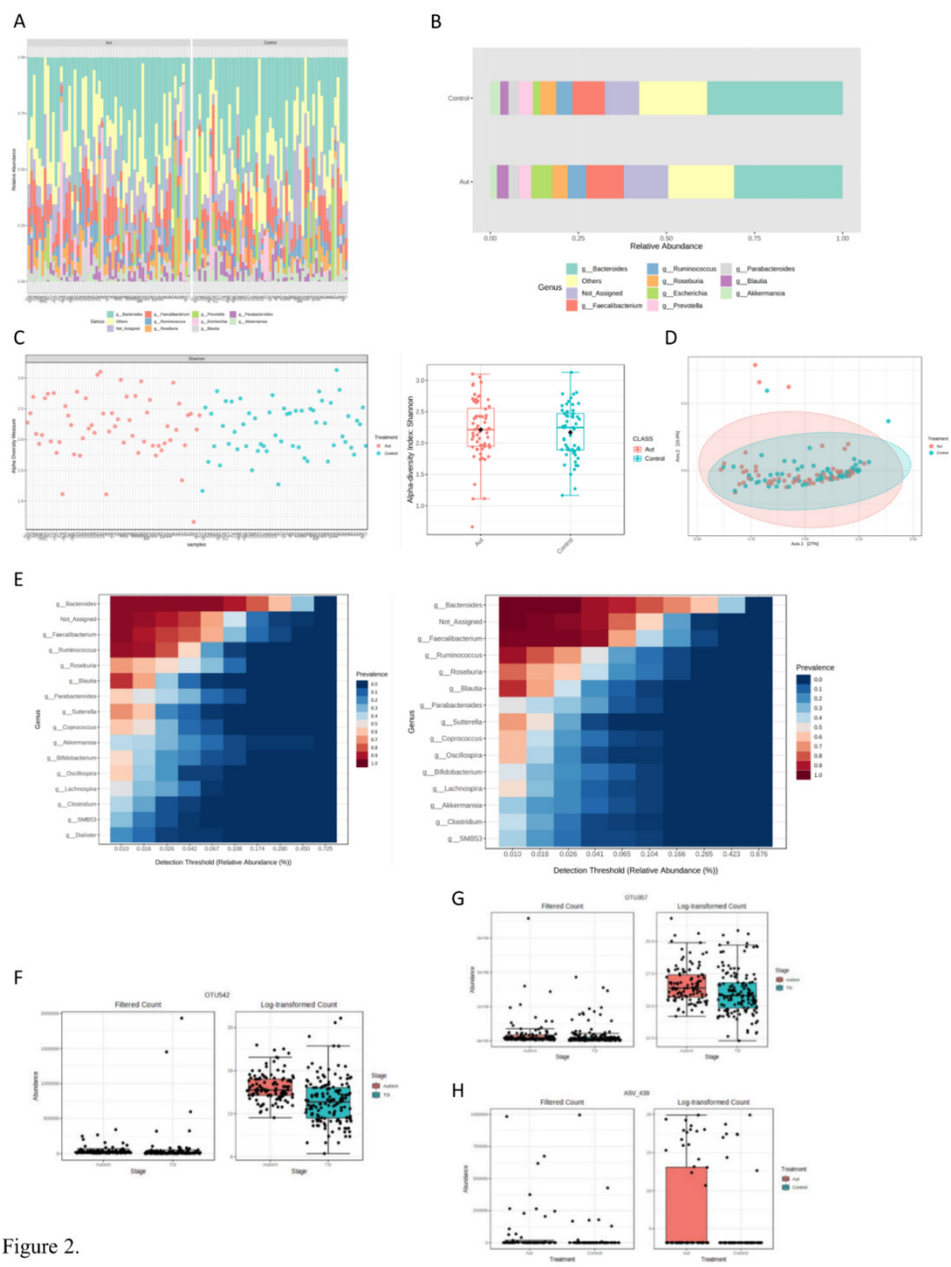

Figure 2.

Figure 2. The microbial communities between age - and sex-matched ASD and healthy children did not show significant differences. (A) Stacked bar analysis (organized samples). (B) Stacked bar analysis (merged samples). (C) Core microbiome analysis (TD group). (D) Alpha-diversity. (E) Beta-diversity. (F) and (G) Differential bacteria from Study 1. (H) Differential bacteria from study 2. (I) Core microbiome analysis (ASD group)

4. Discussion

As depicted in the results, the TD and ASD groups have significant differences in study 1 but not in study 2. The lack of significance in study 2 may be the fact that samples came from siblings and the age difference was less than 2 years. Since diet and lifestyle had a relatively large impact on the gut microbial community, the difference between TD and ASD groups is negligible. We found significant differences in the results in study 1, where the relationship between microbiome and ASD was complex. Many differences were discovered in the TD and ASD groups matched by age and sex, but no significant differences were found in the TD and ASD groups of siblings, which may have been masked by diet and lifestyle.

The study revealed significant differences between the gut microbiomes of ASD and typically developing individuals in study 1, with gender and age not being contributing factors. However, other factors, such as the functional spectrum of prediction, may account for the variations observed in study 2. The absence of significant differences in Study 2 might be attributed to the subjects living together and sharing similar diets, as diet and lifestyle significantly impact the gut microbiome, especially in children under 13 years of age. Therefore, a more comprehensive analysis of the gut microbiome in TD and ASD groups requires consideration of additional data, including diet and lifestyle habits, and the inclusion of larger sample sizes.

It is essential to acknowledge the limitations of sampling data from children, particularly from siblings. Sampling data from adults might yield clearer results and offer additional insights into the role of gut microbiome in ASD and other mental diseases. Regarding the differential bacterium Enterobacteriaceae identified in the LEfSe analysis, its potential secretion of substances related to ASD and other mental diseases warrants further investigation and research (Dinh et al. 2015; He et al. 2023; Liu et al. 2019; Unger et al. 2016). Such findings may hold significant promise for understanding the underlying mechanisms of ASD and developing targeted treatments. Also, the relationship between the gut-brain axis and complex disease may require more clarifications. However, to establish a robust foundation for these findings, future studies should explore the molecular pathways and interactions involving the Enterobacteriaceae family and its potential implications for mental health.

Compliance with ethical standards

This article does not contain any studies with human participants performed by any of the authors.

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