Network-Based Analysis of Gut Microbiome Profiles in Autism Spectrum Disorder

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***Abstract*—The last two decades have witnessed significant increase in research activities associated with Autism Spectrum Disorders (ASD). Many research studies have been conducted to provide better understanding of ASD and attempt to find biological or behavioral signals to help in classification and early detection efforts. In addition, a recent report from the Center of Disease Control (CDC) reported a substantial increase in the percentage of children diagnosed with Autism as compared to the percentage reported a decade earlier. In this study, we explore the possibility of obtaining a biological signal that connects Autism with Gut Microbiome profiles. We employ the concept of similarity networks to analyze micobiome datasets collected in two ASD studies. The key bacterial genera present across two independent datasets are Ruminococcaceae, Fastidiosipila, Firmicutes, Lachnospiraceae, which are consistently associated with variations in microbial community structure. These findings demonstrate the utility of combining similarity networks using cosine similarity and Bray–Curtis dissimilarity to try and identify a unique microbial structure for ASD and controls , which can help us diagnose ASD early on, this study has shown different microbial structure that although are not yet definitive in this preliminary stage, can later be further analyzed to serve as potential biomarkers for early ASD detection and support the development of microbiome-based therapeutic strategies to alleviate symptom severity.**

Keywords-Health Informatics; Autism Spectrum Disorder; Similarity Networks; Biological Markers; Gut Microbiomes Profiles.

I. INTRODUCTION

Concerns over the rising prevalence of individuals being diagnosed with Autism Spectrum Disorder (ASD) have escalated over recent decades. Statistics indicate that almost 1 in every 68 individuals worldwide is diagnosed with ASD [1]. ASD is identified as a persistent deficit in social communication or behavior that can usually be repetitive or restrictive for affected individuals. Diagnostic recategorization and improved screening criteria may be the cause for this surge in numbers, where almost 62.2 million individuals live with ASD, as estimated in 2016 [2]. Numerous studies have highlighted a possible relationship with multiple biomarkers that can aid in detecting ASD early in an individual's life; some of these biomarkers include movement, brain activity, and gut microbiome.

The human gut microbiome comprises a complex population of tens of thousands of microbes. This population includes a variety of organisms, such as bacteria, fungi, archaea, protozoa and viruses [3]. The number of bacteria can vary throughout the gastrointestinal tract, with the colon harboring the highest abundance and most diverse species when compared to the stomach and the small intestine. Hence, the gut microbiomes represent a fundamental role in the host's homeostasis, which is nutrient, immune development, metabolism and defense against pathogens [4]. The gut's microbial balance has been directly linked to human health, where numerous studies have shown that the gut bacteria are directly involved in the fundamental biological processes in humans. Furthermore, the gut microbiome composition can be influenced by the host genetics, diet, age, or external factors, such as geographical location [5]. This study aims to form a relationship between ASD and microbiomes by exploring potential correlations that may act as a diagnostic biomarker.

This paper is comprised of a related work in Section II highlighting the most recent and important findings in the ASD microbiome field related to this paper, followed by a methodology in Section III where the datasets used will be discussed in more details, the metrics used, along with the proposed pipeline and the methodology steps implemented. The results for the implementation will then be presented in Section IV and discussed Section V to highlight the most important findings. Finally, a conclusion for this paper and the future direction of the research will be presented in Section VI.

II. RELATED WORK

In this section a discussion of the most important publications related to the ASD microbiome research are mentioned along with their most important findings.

A review paper by O'Donnell et al. [6] discussed that all species, including animals and humans, have a co-evolving microbial community that colonizes them. However, a short-term diet can have a temporary effect on the gut microbiota; long-term dietary habits can affect the abundance of taxa and have a subsequent impact on the host's health. The most prominent changes can be observed when an individual follows a westernized, fiber-poor diet or a more plant-based, fiber-rich diet, such as the Mediterranean diet. These diets can be associated with higher microbial diversity in the gut.

A paper by Hrnciarova et al. [7] proposed a randomized, double-blinded, placebo-controlled pilot study to evaluate the efficacy of biological response modifier juvenil, which is a mixture of peptides, nucleotides, amino acids, and other components derived from animals, in modulating the microbiome of children with ASD, specifically whether juvenil can help in alleviating the symptoms of ASD. There were 20 ASD and 12 Neurotypical children involved in this pilot study of Czech nationality between the ages of 3 and 7. The samples were collected from each of these children for the duration of 3 months and one group was administered Juvenil, while the other group was administered a placebo.

The results of this pilot study showed that the Juvinel achieved its purpose by making the microbiota of the ASD children quite similar to that of the Neurotypical children. However, it did not result in a complete restoration of the microbiota composition. Furthermore, the behavior of all the ASD children involved in this study was evaluated using CARS2-ST. The group of children who were administered the Juvinel showed a 12.4% reduction in their autism symptoms, which was double that (6.6%) of the placebo group.

Another research by Peralta-Marzal et al. [8] used machine learning (recursive ensemble feature selection) to determine how gut health affects individuals who are diagnosed with ASD, where the primary focus was trying to choose a set of bacterial taxa that defines ASD classification. This was done using sibling-controlled dataset.

The results were obtained by applying RFFS, a method used to discover biomarkers. RFFS is composed of 8 classifiers. This study was successful in identifying 26 bacterial taxa that consistently distinguished ASD from the controls across three independent cohorts, including lifestyle, which influences both ASD and gut microbiome studies. This paper helped prove that there exists a signature for the microbiomes that could identify individuals with ASD from the controls and help reinforce gut health as a valid biomarker.

In a paper published by Taniya et al. [9], several factors were discussed regarding the connection between ASD and the gutbrain axis, mainly the effects of microbiota early dysbiosis in the gestation period, mode of delivery, uncontrolled usage of antibiotics, and stress. These factors affect the gut microbiome and lead to dysbiosis, which then impacts the Central Nervous System function by the production of neurotoxins. The effect of Clostridium found in the colon of children can be used as an indication of ASD development. This paper also discussed the Food and Drug Administration's recognition of microbial transplant therapy as a fast-track treatment for ASD.

This study by Fourquier et al. [10] focused on the gut microbiome of children with ASD in Arizona and Colorado. The results showed differences in microbial structure based on the children's location, suggesting the importance of geographical location when discussing microbiomes. A longitudinal analysis performed linked microbiome shifts with the severity of the displayed ASD behaviors, such as lethargy and inappropriate speech. These findings stress the importance of longitudinal tracking of the ASD subjects, as much more information can still be found from such research.

In a review by Sivamaruthi et al. [11], which again stressed the importance of the gut-brain axis in ASD, we can see how the diet and microbial composition have influenced the symptoms of ASD. This further supports that probiotic supplement can have a powerful potential in improving gut balance, which in turn would help in reducing some of the behavioral symptoms caused by ASD. Though the results still need further clinical trials. Some of the main Risk factors that were stressed in this paper were maternal diet, lifestyle, and early microbial colonization as risk factors that may contribute to ASD development.

From the reviewed studies, it is clear that there is a definitive connection between the gut microbiome and the ASD symptoms which might even allow for the use of the gut microbiome as a biomarker for diagnosis or treatment. It has been shown that clinical interventions, such as dietary modulation, use of biological response modifiers, such as juvenil, and probiotic supplements have shown promise in shifting the microbial profile of ASD individuals with ASD to one that more closely resembles that of a neurotypical individual. Moreover, longitudinal studies have shown that the microbial composition affects not only the diagnosis but also the severity of symptoms of ASD.

In this research, the main aim is to apply a network-based similarity and clustering approach to ASD microbiome datasets. The focus is mainly on the structural properties of microbial communities, not solely on abundance. This can help us make the most of the datasets we can now access. Having access to such datasets will help us build a possible microbial profile that can help in the early identification of ASD in younger individuals.

Improving the overall treatment plan and achieving much better symptom control for individuals.

III. METHODOLOGY

The methodology for the paper will now be presented where the datasets used will be discussed in details, along with the metrics, proposed pipeline and the methodology.

A. Dataset

Multiple 16S rRNA gene sequencing datasets were utilized. These datasets include the taxonomic profiles that were derived from the amplification and sequencing of the phylogenetic marker for the characterization and profiling of microbial communities.

In the Dataset collected by Kang et al. [12], fecal samples were obtained from children between the ages of 4 and 17 years with ASD and neurotypicals to act as controls. The dataset contains 21 neurotypical children and 23 children with ASD. The children did not have a first-degree relationship. Furthermore, the children with ASD were assessed with the Autism Treatment Evaluation Checklist (ATEC) and the Pervasive Development Disorder Behavior Inventory (PDDBI).

The genomic DNA was extracted using a PowerSoil DNA extraction kit, the sequencing analysis was performed using the Quantitative Insights into Microbial Ecology (QIIME). An Operational Taxonomic Unit (OTU) was then obtained in table form by clustering the sequence similarity at 97% where the OTU represents the abundance of each detected bacterium in each sample.

A second dataset (GSE113690) [13] that was utilized in this paper comprises 143 subjects who are clinically diagnosed as ASD, where the sex was male: female 130:13, and the average age was 4.937 ± 0.155. While the Control group had an average age of 5.189 ± 0.170 and a male-to-female ratio of 127:16, 16S rRNA gene sequencing was used in this dataset to evaluate the microbial composition of the ASD and the controls.

Moving on to preprocessing the datasets, a similar structure was followed, where the low-abundance OTUs were removed, meaning the taxa that had near-zero values across most samples were removed. This was done to avoid any bias in the network and to ensure that only the bacteria with any influence remained. For the second dataset, a low-abundance filter had to be applied to ensure that any noise was prevented, which meant eliminating any OUT with counts ≤ 1 in more than 50% of samples.

B. Measures

The measures applied will be the Cosine Similarity and the Bray-Curtis dissimilarity.

1) Cosine similarity

The cosine similarity is a metric that is commonly used to perform tasks, such as information retrieval and data mining.

$$cos(\theta) = \frac{\sum_{i=1}^{d} x_i \cdot x'_i}{\sqrt{\sum_{i=1}^{d} x_i^2} \cdot \sqrt{\sum_{i=1}^{d} x'^2_i}} \quad (1)$$

Cosine similarity focuses more on the direction, where it measures the similarity as the cosine of the angle between two vectors, where two vectors that are considered similar will have a small angle between them [14].
possible link between the microbial composition of individuals with ASD and the individuals who act as controls.

2) Bray-Curtis

The Bray-Curtis index qualifies the dissimilarity between two different taxa over multiple samples. Moreover, BrayCurtis dissimilarity is used to complement the Cosine Similarity; it is used to quantify the compositional difference in the microbiome profile between the ASD and the control Subjects. It focuses on the absolute dissimilarity in the microbiome studies.

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j} \quad (2)$$

Where is the number of samples in which taxa i and taxa j are commonly occurring. and are the number of times that taxa i or taxa j occurred, respectively. If two taxa have a strong relationship, their index will be closer to zero, while if two taxa are entirely independent, they will have an index of 1 [15].

C. Proposed pipeline

This is a proposed general pipeline applied to the two datasets we are currently using in this paper. The pipeline aims to identify any standard microbial profiles that ASD and control cases may share, using these profiles as a biomarker for diagnosing ASD.

D. Methodology steps

In this paper, an attempt is made to construct a similarity structure using the cosine similarity. Cosine similarity assesses the similarity degree between an individual's microbial composition and tries to connect individuals with a similar composition.

1) Data loading and preprocessing

Importing the abundance matrix and its corresponding metadata file allowed for the preprocessing step to occur by replacing any null values with zeros and normalizing the

samples to relative abundance by dividing each taxon count by the total count per sample.

2) Cosine similarity computation

Compute the pairwise cosine similarity between all the sample vectors based on the normalized taxonomic profile. This allowed for a cosine similarity matrix to be generated, where represents the cosine similarity between samples i and j.

3) Graph construction

Building an undirected graph where each node represents a sample. Only the samples with a threshold exceeding 0.75 similarity were included in the graph.

4) Clustering and community detection

Communities were then detected in the constructed graph, identified by exhibiting high microbial similarity and their average relative abundance.

5) *Taxonomy profiling of clusters*

For these clusters, computations were performed to calculate the top-contributing taxa within each cluster using their average relative abundance.

6) Visualization using Bray-Curtis dissimilarity

The Bray-Curtis dissimilarity was then computed to visualize the community differences. The graph was color-coded according to the metadata to differentiate the ASD from the control samples clearly. A heatmap was then generated to illustrate the dissimilarity of the samples.

7) *Analysis of the obtained results*

The analysis of the obtained results was then performed to attempt and identify a microbial profile for ASD individuals and controls.

E. Pseudo code

1) Read the abundance file where rows = sample, cols = taxa.
2) Null values, 0
3) For each sample, divide by row sum so it becomes relative abundance.
4) S = cosine similarity for relative abundance
5) For i<j : if s[i,j] ≥ 0.75 , add weighted edge (I,j,s[I,j]) to graph G.
6) Read metadata
7) Map sample to sample, autistic = red, neurotypical = blue.
8) Draw a graph coloured by group and label nodes with the sample ID.
9) Cluster connected components.
10) For each component k, write the subset of the counts table for its samples to a CSV. 11) BC_Index (relative-abundance, metric is BrayCurtis).
12) Heat-map color by diagnosis.

For dataset loading, the abundance files were the first step, which was then followed by filling all the null values with 0.

Furthermore, all the samples were converted to relative abundance, so all the rows sum to 1. It is then followed by computing the cosine similarity between every pair of samples, which is modeled as an undirected weighted graph, with an edge of similarity only if the pair similarity exceeds 0.75. This threshold allows only the most important relationships to be shown and avoids those that may result in noise.

The metadata is read from its respective file. Each sample is mapped to its metadata to obtain its diagnosis, with autistic samples mapped to the color red and neurotypical samples to the color blue. These samples are then displayed in a weighted graph to visualize the relationship between them. Then each connected component is treated as a cluster, and its data is saved in a CSV file. This is a topology-defined clustering, where if all the members are mutually reachable via over 0.75 edges, then they belong together. The Bray-Curtis index is then used to visualize the data as a heatmap.

IV. RESULTS

Dataset 1, collected by Kang et al. shows several clusters, these clusters seem to have a mixture of both ASD and controls. where the blue nodes signify the controls and the red nodes signify the ASD samples.

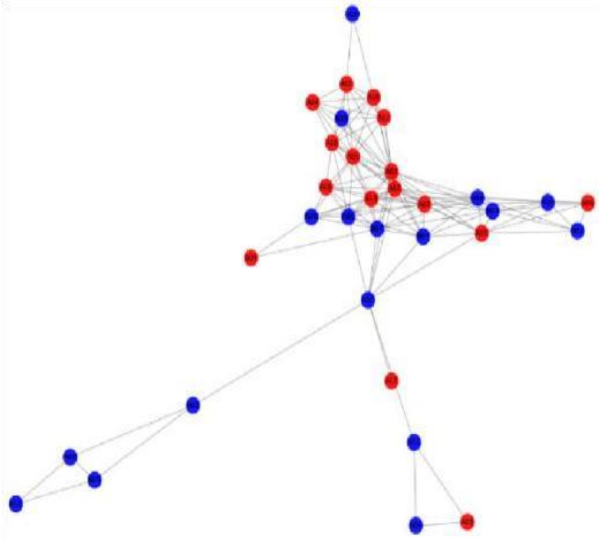

Figure 1. A graph showing the Cosine Similarity undirected graph visualization of samples whose similarity exceeds 0.75 for the first (Kang) dataset.

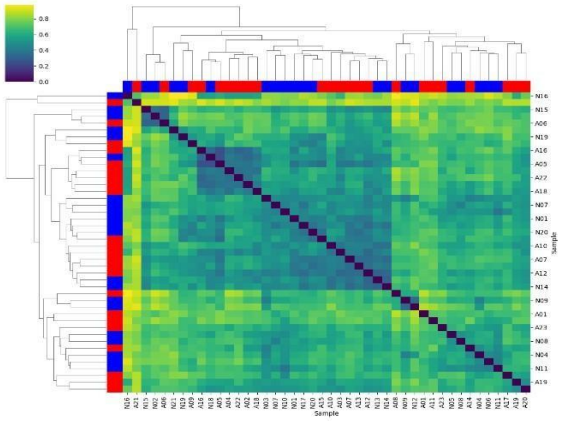

Figure 2. Bray-Curtis dissimilarity heatmap of the first (Kang) dataset.

This dataset provides extensive metadata that can aid in the enrichment analysis. The ATEC and the PDD-BI, which were collected exclusively for individuals with ASD, can help us infer where on the autism scale each individual lies. This will provide clearer information about the microbial structure.

In Figure 1, we can note that there are some dendrogram branches that are dominated by ASD samples (red), while others are dominated by neurotypical samples (blue). Some others have a mixture of both blue and red.

Figure 2 shows the Bray-Curtis dissimilarity of the Kang dataset, where the red indicates the ASD samples and the Blue indicates the neurotypical samples. This was done to help visualize the diagnosis of the samples. For the BrayCurtis dissimilarity, the lower values and darker colors mean that these samples share a similar microbial composition, while the higher values with more yellowish hues indicate greater differences. While the second dataset, as shown in Figure 3, does not show a clear separation, but we can still infer some information from its microbial structure, since this dataset doesn't provide much metadata according to the severity of the ASD.

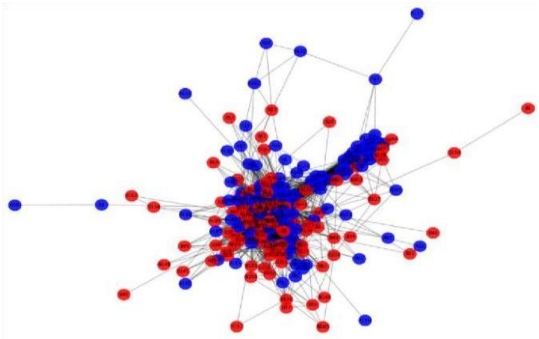

Figure 3. shows the Cosine Similarity undirected graph visualization of samples whose similarity exceeds 0.75 for the second dataset (GSE113690).

Furthermore, using the Bayes-Curtis dissimilarity shown in Figure 4, we can see that the heat map provides us with a more understandable view of the top 100 samples. Most values in this heatmap have an intermediate range.

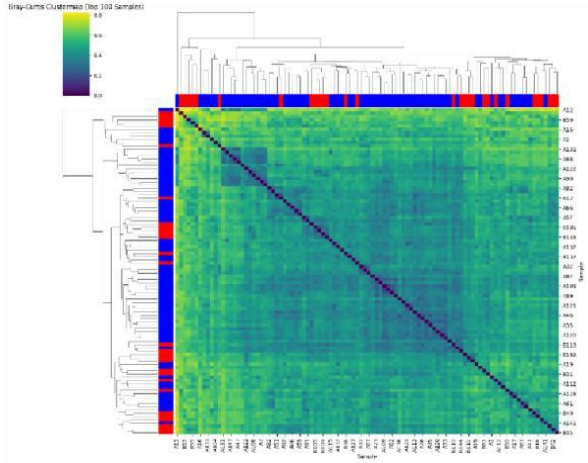

Figure 4. Bray-Curtis dissimilarity heatmap for the second (GSE113690) dataset.

The colors indicate a mixed but uneven distribution, with red samples representing ASD individuals and blue samples representing neurotypical individuals

V. DISCUSSIONS

The analysis of Figure 1 shows some promising microbial structures detected, which show us what a typical gut microbiome diversity looks like. We had 3 clusters where the first cluster had an almost even distribution of ASD and control samples, with 21 control samples and 21 ASD samples, where the most prominent bacteria profile with a mean relative abundance of 0.158, where the most common were Bacteroidota, Bacteroidia, Bacteroidales, Bacteroidaceae, Phocaeicola.

Following through with the second cluster had only one ASD, the sample had a mean relative abundance of 0.298, with the most common bacteria being Firmicutes, Clostridia, Lachnospirales, Lachnospiraceae, Hungatella. Finally, the third cluster has one ASD sample with its mean relative abundance being 0.669 and the most prominate bacteria being Verrucomicrobiota, Verrucomicrobiae, Verrucomicrobiales, Akkermansiaceae, Akkermansia.

Taking a deeper look into these results we can see that in the first cluster there is equal samples of control and ASD, where the most prominent bacteria found can be an indication of a balanced gut barrier and a strong gut barrier when they are present in moderation, but if there is an imbalance or over abundance, this can show various health issues. While for the second cluster the most prominent microbial profile can indicate a healthy and diverse gut eco system, yet Hungatella ‘s abundance may have a potential role in diseases. Moving on to the third cluster where the

presence of this microbial profile can indicate a reduction in the gut inflammation.

Using a genus-level cosine network on the (GSE113690) balanced dataset, we can identify 6 main clusters, where for the first cluster had 43 ASD samples and 47 controls and a mean relative abundance of 0.125, which the most common bacteria being Firmicutes, Clostridia, Clostridiales, Ruminococcaceae, which can have an effect on cognitive function, Fastidiosipila, Clostridiales_bacterium.

The second cluster had 34 ASD samples and 31 controls with a mean relative abundance of 0.104 where the most common bacteria were Proteobacteria, Alphaproteobacteria, Rickettsiales.

The third cluster is comprised of 12 ASD samples and 35 Controls with a mean relative abundance of 0.202 with the most common bacteria being Firmicutes, Clostridia, Clostridiales, Lachnospiraceae, Tyzzerella.

The fourth cluster has 14 ASD samples and 16 Control samples, with a mean relative abundance of 0.079 with the most common bacteria being Firmicutes, Clostridia, Clostridiales, Ruminococcaceae, Fastidiosipila.

The fifth cluster had an equal amount of ASD and Controls with 2 samples each, a mean relative abundance of 0.103 and the most common bacteria being Firmicutes, Clostridia, Clostridiales, Ruminococcaceae, Fastidiosipila. Finally, the sixth cluster has 2 control samples with a relative abundance of 0.116 and the most common bacteria being Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, Perlucidibaca.

For the first cluster, the bacterial profile is essential to maintain a healthy gut and strengthening the gut barrier. While for the second cluster, has a Rickettsiales , which is considered a pathogen that is responsible for multiple diseases, such as Rocky Mountain spotted fever or typhus [16]. For the third cluster we can see that that the controls are much more abundant where Tyzzerella can act as a biomarker for certain conditions, such as Clostridioides difficile infection. For the fourth cluster and fifth cluster, we can see a similar microbial profile, where we can see that both almost has an equal sample of ASD and controls inside them. where Firmicutes is one of the most abundant bacteria in the human gut. Finally for the sixth cluster, it has Perlucidibaca, which is not typically considered a resident of human gut microbiome.

To conclude this discussion, a gut microbial profile for either, the ASD or the Controls, cannot be deduced at this point, but some promising microbial structures have been detected, which show us what a typical gut microbiome diversity looks like. These results are not definitive yet at this state to act as a biomarker, but they are definitely a step in the right direction to help build a separate microbial profile for controls and ASDs.

VI. CONCLUSION AND FUTURE WORK

The results are still in the early stages and not clear microbial profile has been identified at this point in this ongoing research. Different profiles have been identified, which can benefit from further research to be able to pinpoint how each bacterium in a certain profile interact together, which can show us how healthy the gut microbiome really is, contributing to being able to build a solid microbial profile for ASD individuals. The current datasets were not able to provide us with severity indication, and have shown very similar structures for both the ASD and the controls, proving that both profiles do share a certain degree of similarity in their microbial profile, but at this point the exact difference between such profiles cannot be determined.

Most of the profiles detected are normal occurrences in a healthy gut microbial structure, so further studies will need to be implemented to be able to extract distinct gut microbial profiles that will help us in Diagnosing ASD early on.

From childbirth, a steady interaction is formed between the human body and its environmental microbial structure [18]. To pinpoint the exact effect of different environments on the gut microbiota, a larger sample of individuals will need to be studied over a long period to determine the environmental impact on the gut microbiome of any individual. This will be able to show the effect of the environment, the impact of the diet, and the exposure to medication and antibiotics [19]. It has been proven that the gut microbiota can be affected by antibiotic intake, where antibiotic prescriptions can be a viable solution to treat some more prominent ASD symptoms, by using these prescriptions to alter their microbiota to match that of more neurotypical individuals [20].

Furthermore, using a multi-cohort dataset can prove to be a beneficial way to try and gather a larger dataset. In addition, combining multiple biomarkers can help in detecting ASD symptoms earlier on and more efficiently, as it will help detect ASD at an earlier age, which in turn will help alleviate some of the severe symptoms that can be treated if detected early on.

Finally, more metadata should be collected about the samples, since ASD is not only related to the physical symptoms, but the attitude and the behavior of the diagnosed individual can act as an indicator that can help diagnose ASD earlier. A more refined dataset with not only microbiome data, but also combined with behavior and attitude of the affected individuals and controls can provide for useful for more extensive research in ASD biomarkers. These Datasets can help us understand and classify the severity of the ASD diagnosis and treat the patient accordingly.

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