Unlocking the Microbial Mysteries in Children with Special Needs: A High School Journey Through the Gut

Grades 9 – 12 Edition

By
Trisha Raichura[A], Anitha Sundararajan[B], Joann Mudge[C], and Thiruvarangan Ramaraj[D]

[A]Dubai College, Dubai, UAE
[B]University of Chicago, Chicago, IL, USA
[C]National Center of Genome Resources, Santa Fe, NM, USA
[D]DePaul University, Chicago, IL, USA

Introduction.

Autism spectrum disorder is a developmental disability caused by differences in the brain. People with autism can have restricted behavior, problems with communication/paying attention or even physical learning challenges. There are a wide range of conditions in autism spectrum disorder, which affects 1 in 44 children today. Autism symptoms, especially with more advanced cases, appear around the ages of 12-14 months or earlier.

Objectives.

Student Background Knowledge.

Part I. Domains of life. The three domains of life are the Archaea, the Bacteria and the Eukarya. A microbiome is the community of microorganisms that can usually be found living together in any given habitat. Microorganisms from all three domains are present in the human gut microbiome. However, most gut microbiome species are bacterial. These members of the domain Bacteria and the kingdom Eubacteria are unicellular and prokaryotic.

Part II. Gut microbiota.

Gut microbiota are microorganisms that live in the digestive tracts of vertebrates and invertebrates. These bacteria and other types of microorganisms help with digestion, destroy harmful bacteria and help control the immune system. The microorganisms can be both helpful and potentially harmful. Most of the microorganisms that take up residence in the human gut have symbiotic relationships with the human body where they both the microorganism and humans benefit. However, a few are pathogenic, meaning they spread or carry disease. Even though these pathogenic microorganisms exist, in a healthy human body both symbiotic and pathenogenic bacteria coexist problem-free.

Benefits to having microbiome:

- Stimulates the immune system, meaning they aid in the breakdown of food compounds and synthesize certain vitamins and amino acids.
- Smaller compounds like table sugar and lactose can be absorbed quickly in the small intestine, microbiota help break down larger particles such as complex carbohydrates and starch with their digestive enzymes.

An imbalance of unhealthy and healthy gut microbiota can contribute to weight gain, high blood sugar, high cholesterol and other disorders.

Apart from genetic factors, it has been suggested that the gut microbiome may have a part to play in autism spectrum disorder. And the evidence suggests that the pathway between gut bacteria and the central nervous system, referred to as the gut–brain axis, has a profound effect on social behaviors.

Part III. DNA Sequencing.

Sequencing DNA means determining the order of the four chemical building blocks - called "bases" - that make up the DNA molecule. The sequence tells scientists the kind of genetic information that is carried in a particular DNA segment. For example, scientists can use sequence information to determine which stretches of DNA contain genes and which stretches carry regulatory instructions, turning genes on or off. In addition, and importantly, sequence data can highlight changes in a gene that may cause disease.

In the DNA double helix, the four chemical bases always bond with the same partner to form "base pairs." Adenine (A) always pairs with thymine (T); cytosine (C) always pairs with guanine (G). This pairing is the basis for the mechanism by which DNA molecules are copied when cells divide, and the pairing also underlies the methods by which most DNA sequencing experiments are done. The human genome contains about 3 billion base pairs that spell out the instructions for making and maintaining a human being.

Part IV. 16S rRNA Gene.

16S rRNA gene is used as a marker for identification and taxonomic classification of microbes. Since this gene is ubiquitous and highly conserved among bacteria as well as archaea, it is popularly used for phylogenetic studies.

Part V. 16S rRNA Sequencing.

The 16S rRNA gene consists of both conserved and variable regions. While the conserved region makes universal amplification possible, sequencing the variable regions allows discrimination between specific different microorganisms such as bacteria, archaea and microbial eukarya. Identification of viruses requires metagenomic sequencing (the direct sequencing of the total

DNA extracted from a microbial community) due to their lack of the phylogenetic marker gene 16S.

Conserved- present in everything - not studied.
Hypervariable region- part that is studied.
Primers - enable you to read through hypervariable regions- present in both directions.

Part VI. Gut Microbiome and Autism Spectrum Disorder.

Research into the relationship between the gut microbiome and autism spectrum disorder (ASD) is an emerging and evolving field. While there is no conclusive evidence that links gut microbiota directly to the development of ASD, there is growing interest in understanding the potential connections between the two. Here are some key points and findings regarding the gut microbiome and ASD:

1. Gut-Brain Axis: The gut-brain axis is a bi-directional communication system between the gut and the brain. Emerging research suggests that the composition of the gut microbiota may influence brain function and behavior. Alterations in the gut microbiome could potentially impact neurological and behavioral development, which is relevant to ASD.

2. Microbiome Composition: Some studies have found differences in the gut microbiota of individuals with ASD compared to neurotypical individuals. These differences often involve changes in the relative abundance of certain bacterial species. However, these findings are not consistent across all studies, and there is a need for more research to establish clear patterns.

3. Potential Mechanisms: Several potential mechanisms have been proposed to explain how gut microbiota might influence ASD. These include the production of metabolites (such as short-chain fatty acids) that can affect the central nervous system, the modulation of the immune system, and the influence on the production of neurotransmitters.

4. Immune System: The gut microbiome plays a crucial role in the development and maintenance of the immune system. Dysregulation of the immune system has been observed in some individuals with ASD, and there is evidence to suggest that the gut microbiota can influence immune function.

5. Clinical Implications: While the link between gut microbiota and ASD is still not fully understood, some researchers and clinicians have explored potential dietary and probiotic interventions to modulate the gut microbiome as a complementary approach to managing certain symptoms of ASD. However, the effectiveness of these interventions remains a subject of ongoing investigation.

6. Limitations: It's essential to recognize that the research on the gut microbiome and ASD is in its early stages. Many studies are small and may have methodological limitations, making it challenging to draw definitive conclusions.

In summary, there is growing interest in the potential role of the gut microbiome in ASD. While some research suggests there may be associations between gut microbiota composition and ASD, more extensive and rigorous studies are needed to establish the nature and mechanisms of this relationship.

Vocabulary

Materials Checklist

Access to a laptop or desktop computer.
Access to datasets

Classroom Management

Overview.
Pre-class preparation.
Powerpoint presentation.

Blocks of Analysis

The analysis was reproduced using QIIME2 on a UNIX server. Students will be provided accounts on the server and work through the commands provided in the PDF tutorial.

Alternatively, the analysis will be on a galaxy web server which be ideal for classroom settings. Efforts are underway to implement the analysis on a web server.

Sample Questions

1) What do the different colors in the bar plots mean?
2) What are the differences between healthy patients and compromised patient samples?
3) Describe what is on the x and y axis

QIIME2 Analysis

2023-10-09

```
qiime tools import --type "SampleData[SequencesWithQuality]" \
--input-format SingleEndFastqManifestPhred33V2 \
--input-path ./manifest.tsv \
--output-path ./demux_seqs.qza
```

```
qiime demux summarize \
--i-data ./demux_seqs.qza \
--o-visualization ./demux_seqs.qzv
```

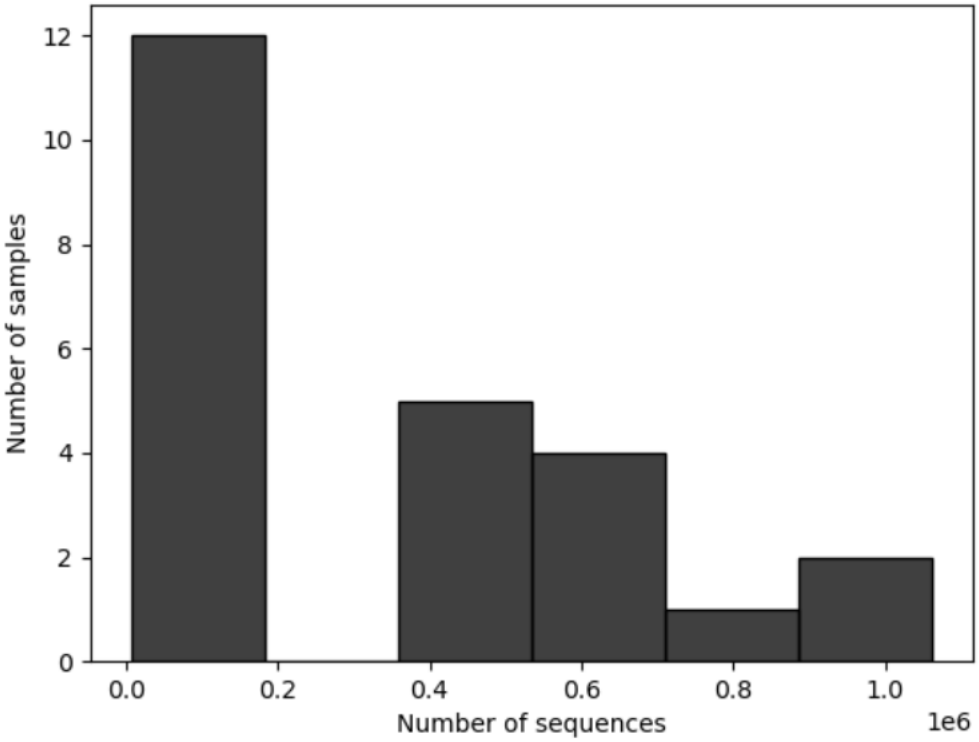

Figure 1: Figure1: Sequence Reads Frequency Histogram

```
qiime dada2 denoise-single \
--i-demultiplexed-seqs ./demux_seqs.qza \
--p-trunc-len 150 \
--o-table ./dada2_table.qza \
--o-representative-sequences ./dada2_rep_set.qza \
--o-denoising-stats ./dada2_stats.qza
```

```
qiime metadata tabulate \
--m-input-file ./dada2_stats.qza \
--o-visualization ./dada2_stats.qzv
```

```
qiime feature-table summarize \
--i-table ./dada2_table.qza \
--m-sample-metadata-file ./metadata.tsv \
--o-visualization ./dada2_table.qzv
```

```
wget  https://data.qiime2.org/2023.7/common/gg-13-8-99-515-806-nb-classifier.qza
```

```
qiime feature-classifier classify-sklearn \
--i-reads ./dada2_rep_set.qza \
--i-classifier ./gg-13-8-99-515-806-nb-classifier.qza \
--o-classification ./taxonomy.qza
```

```
qiime metadata tabulate \
--m-input-file ./taxonomy.qza \
--o-visualization ./taxonomy.qzv
```

```
qiime feature-table tabulate-seqs \
--i-data ./dada2_rep_set.qza \
--o-visualization ./dada2_rep_set.qzv
```

```
qiime feature-table filter-samples \
--i-table ./dada2_table.qza \
--p-min-frequency 2000 \
--o-filtered-table ./table_2k.qza
```

```
qiime taxa barplot \
--i-table ./table_2k.qza \
--i-taxonomy ./taxonomy.qza \
--m-metadata-file ./metadata.tsv \
--o-visualization ./taxa_barplot.qzv
```

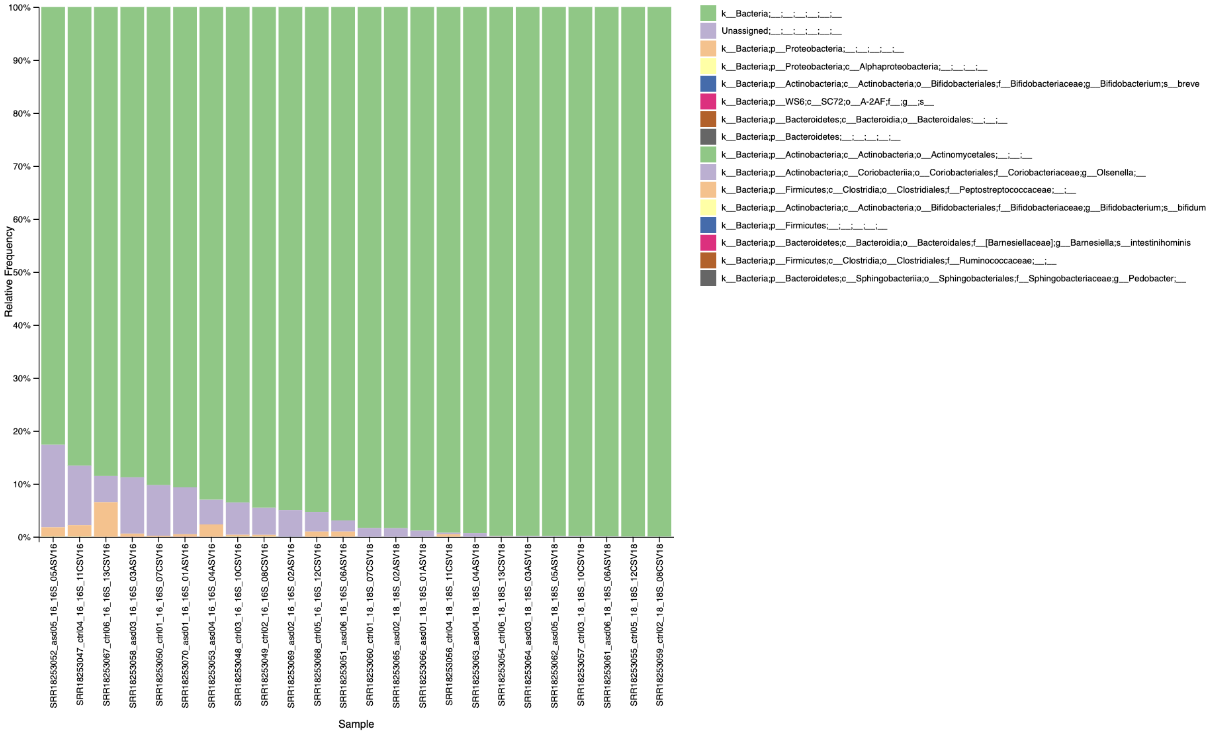

Figure 2: Figure2: Taxonomy Bar Plots at species level