Age-Specific Gut Microbiota Dysbiosis in Autoimmune Disease: A Comparative Analysis Between Pediatric and Adult Cohorts

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Abstract: ***Gut microbiota plays a fundamental role in regulating immune function and has been increasingly linked to the development of autoimmune diseases. This study compares the gut microbial composition and diversity in children and adults with autoimmune disorders to those of healthy controls. Stool samples were examined through 16S rRNA gene sequencing to assess microbial diversity, relative abundance of key taxa, and functional pathways potentially involved in immune dysregulation. The analysis revealed a marked decline in overall microbial diversity and notable alterations in bacterial groups, particularly Firmicute and Bacteroidetes, among patients with autoimmune conditions. Distinct age- related patterns were observed, underscoring differences in microbial signatures between children and adults. These findings highlight the crucial role of gut microbiota in autoimmune pathogenesis and suggest the potential of age-specific microbiota-targeted interventions. Continued longitudinal and mechanistic research is required to translate these insights into effective clinical application.***

I. INTRODUCTION

The human gut microbiota, comprising trillions of microorganisms such as bacteria, archaea, viruses, and fungi, plays a crucial role in maintaining host health through its involvement in digestion, metabolism, and immune regulation (Sommer & Bäckhed, 2013). Increasing evidence indicates that disturbances in this microbial ecosystem—commonly referred to as gut dysbiosis—are closely linked to the development of autoimmune diseases, including Type 1 Diabetes (T1D), Rheumatoid Arthritis (RA), and Multiple Sclerosis (MS) (Zhou et al., 2021). Autoimmune diseases arise when the immune system mistakenly targets the body's own tissues, resulting in chronic inflammation and progressive tissue damage. While genetic predisposition is a recognized determinant of disease risk, environmental influences such as diet, infections, and, most notably, gut microbiota, have emerged as key contributors to the initiation and progression of autoimmunity (Belkaid & Hand, 2014).Notably, interactions between gut microbiota and the immune system vary across the human lifespan, making it essential to explore how these dynamics differ between children and adults with autoimmune conditions. In early life, the immune system is still developing. In contrast, adults generally exhibit more stable yet altered microbiota profiles, which are further influenced by lifestyle factors and aging (O'Toole & Jeffery, 2015). Identifying these age-specific microbial signatures is therefore crucial for developing tailored microbiome-based strategies to prevent or modulate autoimmune diseases.

This study investigates and compares the gut microbiota profiles of children and adults with autoimmune diseases with those of healthy controls. By integrating microbial diversity analyses and taxonomic profiling, the study seeks to uncover age- dependent microbial patterns associated with autoimmunity and evaluate their potential mechanistic roles and therapeutic implications.

II. LITERATURE SURVEY

The human gut microbiota is a complex ecosystem of trillions of microorganisms—including bacteria, archaea, viruses, and fungi—that profoundly influence host physiology, metabolism, and immunity. A balanced microbiota is essential for health, while disruptions, or dysbiosis, have been strongly associated with autoimmune diseases such as Type 1 Diabetes (T1D), Rheumatoid Arthritis (RA), and Multiple Sclerosis (MS). These conditions often involve reduced microbial diversity and notable shifts in key groups like

Firmicutes and Bacteroidetes, which regulate immune tolerance and inflammatory pathways.

Age is a critical determinant of microbial composition. Early-life microbial exposures play a central role in immune maturation, while adult microbiota, though relatively stable, are shaped by diet, lifestyle, antibiotics, and environment. Studies consistently show that children and adults with autoimmune conditions exhibit distinct microbial patterns, reflecting differences in immune development and environmental interactions.

High-throughput sequencing technologies, especially 16S rRNA gene sequencing, have transformed microbiome research, enabling detailed profiling of microbial communities.

Computational bioinformatics, particularly Python-based workflows, is now indispensable for handling large datasets. Through the generation of OTU (Operational Taxonomic Unit), ASV (Amplicon Sequence Variant), metadata, and taxonomy tables, researchers can classify microbes, compare groups, and uncover disease-associated signatures with high precision.

Building on this foundation, the present study integrates sequencing data with computational analysis to compare gut microbiota between children and adults with autoimmune diseases and healthy controls. By applying Python-based frameworks to construct OTU, ASV, metadata, and taxonomy tables, the study aims to reveal age-specific microbial signatures, explore mechanisms underlying autoimmunity,

By applying Python-based frameworks to construct OTU, ASV, metadata, and taxonomy tables, the study aims to reveal age-specific microbial signatures, explore mechanisms underlying autoimmunity, and highlight microbiota-targeted strategies. This structured approach enhances reproducibility and underscores the pivotal role of bioinformatics in advancing microbiome research.

III. PROPOSED SYSTEM

This study proposes a coding-driven framework to investigate gut microbiota in children and adults with autoimmune diseases compared to healthy controls. Stool samples will be collected with ethical approval, and 16S rRNA sequencing will be applied to characterize microbial communities. Metadata such as age, sex, disease type, clinical history, and lifestyle factors will be systematically recorded to enable meaningful age-specific comparisons.

The sequencing datasets will be processed through Python- bioinformatics workflows using tools such as QIIME2, DADA2, and scikit-bio. After quality control and denoising, structured OTU, ASV, metadata, and taxonomy tables will be generated. These outputs will form the foundation for microbial diversity assessments, taxonomic profiling, and disease-specific biomarker discovery. Machine learning models implemented in Python will further classify microbial patterns and highlight taxa associated and autoimmunity, while adjusting for confounders such as diet and antibiotic exposure.

This proposed system ensures reproducibility through open- source coding pipelines and standardized data handling. By combining sequencing with computational analysis, it establishes a robust framework to uncover age-specific microbial-signatures, explore mechanisms contributing to autoimmune diseases, and support future microbiota-based diagnostic and therapeutic strategies.

IV. RESULT

GUT MICROBIOTA

Sample ID	Age	Sex	Value 1	Value 2	Disease Status
S001	34	M	30%	60%	Healthy
S002	45	F	40%	45%	Moderate Dysbiosis
S003	52	M	25%	55%	High Dysbiosis
S004	50	F	32%	30%	Healthy
S005	40	F	38%	50%	Moderate Dysbiosis
S006	32	F	32%	29%	Healthy
S007	50	M	30%	30%	Healthy
S008	40	F	32%	25%	Healthy
S010	40	F	36%	50%	Healthy
S011	38	F	32%	30%	Healthy
S012	30	F	32%	25%	Moderate Dysbiosis
S013	26	M	25%	18%	High Dysbiosis
S014	25	F	18%	13%	Healthy
S015	30	M	36%	30%	Healthy
S016	30	F	30%	25%	Healthy
S017	28	M	30%	30%	Healthy
S020	38	M	45%	30%	Moderate Dysbiosis

Figure 1: Result Dataset

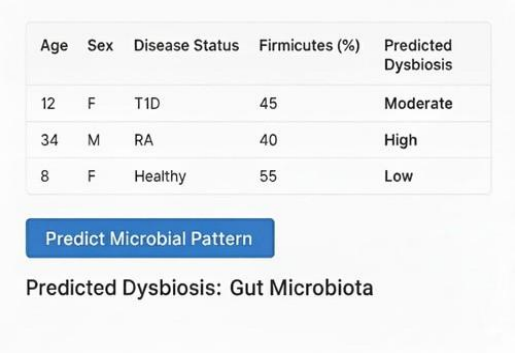


Figure 2: Predict Dysbiosis

V. METHODOLOGY

1. Sample Collection
   - Participant recruitment: Individuals were recruited based on age, sex, and clinical health status, categorized as healthy, moderate diagnosis, or high diagnosis.
   - Stool collection: Fecal samples were collected in sterile, DNA-free containers following standard biosafety protocols.
   - Storage and transport: Samples were immediately stored at −80°C and transported on dry ice to preserve microbial integrity.

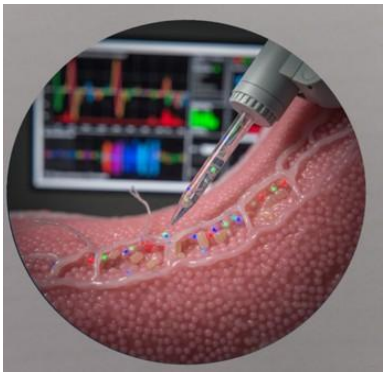
2. DNA extraction: Microbial DNA was extracted using a commercially available kit, following the manufacturer's protocol.
   - Quality assessment: DNA concentration and purity were measured using spectrophotometry and fluorometric quantification.
   - 16S rRNA gene sequencing: The V3–V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina platform for bacterial profiling.

3. Data Processing and Taxonomic Assignment
   - Quality control: Raw sequences were filtered to remove low-quality reads, chimeric sequences, and contaminants.
   - OTU/ASV generation: Sequences were clustered into Operational Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASVs) with 97% similarity.
   - Taxonomic classification: OTUs/ASVs were assigned taxonomy using the SILVA and Greengenes reference databases.

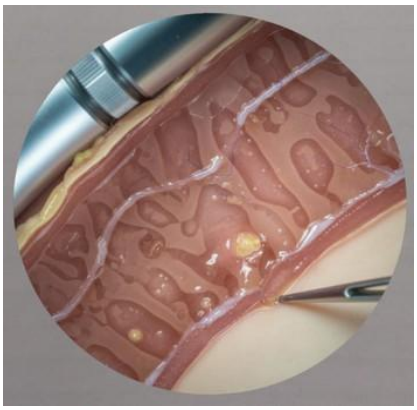
4. Microbiota Analysis and Dysbiosis Prediction
   - Relative abundance: The proportional representation of major bacterial phyla, such as Firmicutes and Bacteroidetes, was calculated.
   - Diversity analysis: Alpha diversity (within- sample diversity) and beta diversity (between- sample differences) were computed using standard ecological indices.
   - Predicted dysbiosis: A machine learning-based model was employed to classify samples as healthy, moderate, or high dysbiosis based on microbial composition.

5. Statistical Analysis and Interpretation
   - Correlation analysis: Associations between microbial composition and clinical variables (age, sex, disease status) were assessed using appropriate statistical tests.
   - Data visualization: Heatmaps, bar plots, and principal coordinate analysis (PCoA) plots were generated to illustrate microbial diversity and dysbiosis patterns.
   - Interpretation: Key altered taxa were identified, and the potential implications of microbial imbalance for host health were discussed.

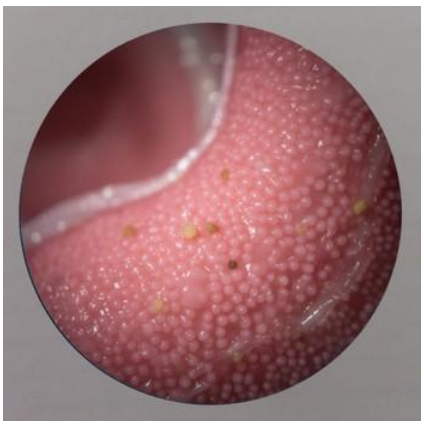
"Shows lab processing for 16S rRNA sequencing, generating data for bioinformatics insights on microbial composition and function."



Depicts genetic extraction and bioinformatics in microbial analysis, highlighting gut microbiota profiling and disease- specific insights."



″Shows the gut's microscopic environment, where host cells and microbes interact, key to autoimmune insights."



″Illustrates gut structure with areas of inflammation and microbial activity, emphasizing the context of dysbiosis."

VI. CONCLUSION AND FUTURE WORKS

The present study demonstrates that gut microbiota profiling, combined with computational and machine learning approaches, offers a powerful tool for the detection and prediction of dysbiosis. Analysis of microbial composition and diversity enables the identification of specific bacterial patterns associated with health status, providing valuable insights into host-microbiota interactions. These findings highlight the potential of gut microbiota as a non-invasive biomarker for monitoring health and disease progression.

Future work can focus on the development of predictive models that not only classify dysbiosis levels—healthy, moderate, or high—but also identify key microbial taxa linked to disease risk and progression. Integrating multi- omics datasets, conducting longitudinal studies, and including larger, more diverse cohorts can enhance the accuracy and clinical relevance of these models. Such advancements may ultimately support personalized interventions and therapeutic strategies aimed at restoring microbial balance and promoting overall health.

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