# Metabolomic Insights: Unraveling the Evolution of Short-Chain Fatty Acids Levels in Autism Spectrum Disorder Onset

**Alessio Trotta[1,2*], Martina Lombardi[1,2,3], Vera Abenante[1,2], Nicole Palmieri[1,2,3], Andrea Ingenito[1,2] and Jacopo Troisi[1,2,4]**

1.Theoreo srl, Via degli Ulivi 3, 84090, Montecorvino Pugliano, SA, Italy; 2.European Institute of Metabolomics - Fondazione EIM, Via Puccini 2, 84081, Baronissi, SA, Italy; 3. Department of Chemistry and Biology, "A. Zambelli", University of Salerno, Fisciano, SA, Italy; 4.Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Baronissi, SA, Italy;

## Introduction

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition marked by a wide array of symptoms and clinical features. Recently, the significance of the gut microbiome in ASD has drawn growing scientific interest. Numerous studies have shown a link between changes in the gut microbiome and the onset and severity of ASD symptoms. The GEMMA project, which stands for Genome, Environment, Microbiome, and Metabolome in Autism, seeks to assess how external factors affect the gut microbiota and how interactions between the genome and metagenome can trigger an immune response due to significant epigenetic and metabolomic changes. These changes may facilitate and increase molecular traffic in the gut, contributing to the onset of ASD. In this context, analyzing levels of Short-Chain Fatty Acids (SCFAs), key microbial metabolites in the gut microbiome, has emerged as a promising research direction to better understand the relationship between the gut microbiome and ASD. SCFAs, produced through microbial fermentation of dietary fibers in the colon, are crucial for regulating intestinal inflammation, maintaining the mucosal barrier, and facilitating gut-brain interactions—all processes implicated in ASD pathophysiology. By conducting longitudinal analyses of SCFA levels in individuals at risk of developing ASD, we aim to identify potential metabolic patterns associated with the onset and progression of ASD. The results of this study could provide significant insights into the underlying mechanisms of ASD and pave the way for developing targeted diagnostic and therapeutic strategies.

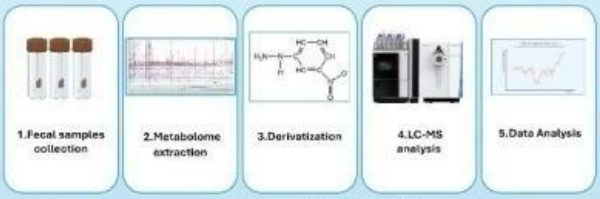


*Figure 1: SCFAs analysis pipeline*

## Materials and Methods

Fecal samples, were collected from 40 children that met the criteria for ASD diagnosis according to the BOSA criterium and 32 children, chosen as matched healthy controls.

These samples were subjected to extraction using the IPA (cold isopropanol), and derivatization using 3-nitrophenylhydrazine. This method has the advantages over other techniques especially for its high level of selectivity, reproducibility, and robustness for SCFAs quantification. The resulting solution were then analyzed using a targeted metabolomics approach employing Liquid Chromatography-Mass Spectrometry, focusing on SCFAs levels. Specifically, Vanquish HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) comprises a column compartment (VC-C10-A), an automated sampler (CV-A12-A), and a binary pump (CV-P10-A), utilizing a Kinetex Evo C18 column with dimensions of 150 mm x 2.1 mm and a particle size of 2.6 µm, and a pore size of 130 Å (Phenomenex, Torrance, CA, USA). The mobile phase A (MPA) is a solution of 0.1% formic acid (VWR, Avantor, Radnor, PA, USA) in ultrapure water (VWR, Avantor, Radnor, PA, USA), while the mobile phase B (MPB) consists of 0.1% formic acid in methanol (VWR, Avantor, Radnor, PA, USA). The chromatographic separation began at a flow rate of 0.35 mL/min with 20% MPB, maintained for 2 minutes, then adjusted to 55% MPB over 8 minutes, and increased to 99% MPB at 8.1 minutes. This condition was held until 9.1 minutes, followed by a reversion to 20% MPB at 9.5 minutes, maintaining this for 1 minute. The flow rate was increased to 0.4 mL/min at 8.1 minutes to facilitate the removal of less polar contaminants, then returned to the initial rate. The column temperature was consistently maintained at 40 °C with an injection loop volume of 2 µL. Detection of short-chain fatty acids (SCFAs) was conducted using an Orbitrap Exploris™ 120 Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in negative ESI mode. Preliminary individual analyses for each SCFA were performed to determine retention times and mass fragmentation patterns. The mass spectrometer operated at 2500 V in static gas mode, utilizing 45 arbitrary units of sheath gas and 12 units of auxiliary gas. The Ion Transfer Tube was set to 290 °C, and the Vaporizer Temperature was maintained at 320 °C. Two MS segments were employed: the first segment involved a full scan analysis (100-1000 m/z, 120,000 resolution) with an RF level of 70%, while the second segment was an MS2 scan with a 30% HCD collision energy and a resolution of 15,000, using an automatic scan range. Quantification of all SCFAs was performed using a calibration curve based on 10 calibration points, each analyzed in triplicate as depicted in Fig. 2. The SCFAs were confirmed as derivatized adducts by evaluating both the expected exact mass and the presence of a 137.04 fragment.

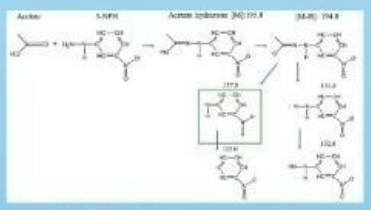

*Figure 2: SCFAs derivatization. Acetate was used as an example*

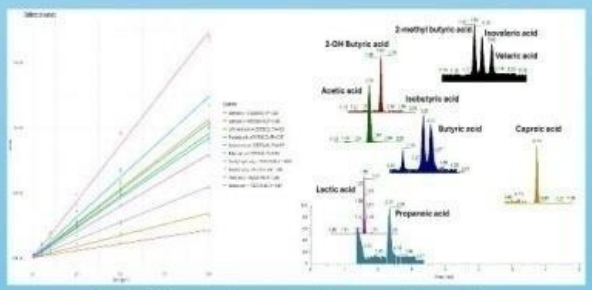


*Figure 3: SCFAs calibration curves and chromatographic separation*

## Results

The outcomes of our research highlight a significant difference in the longitudinal development of certain SCFA profiles between children with ASD and those without. This difference is most notable in Acetic Acid, Hexanoic Acid, Hydroxybutyric Acid, and Valeric Acid. These variations indicate a possible role of the gut microbiome in the onset and progression of ASD, suggesting changes either in microbial composition or in the metabolic activities within the gut environment of children with ASD.

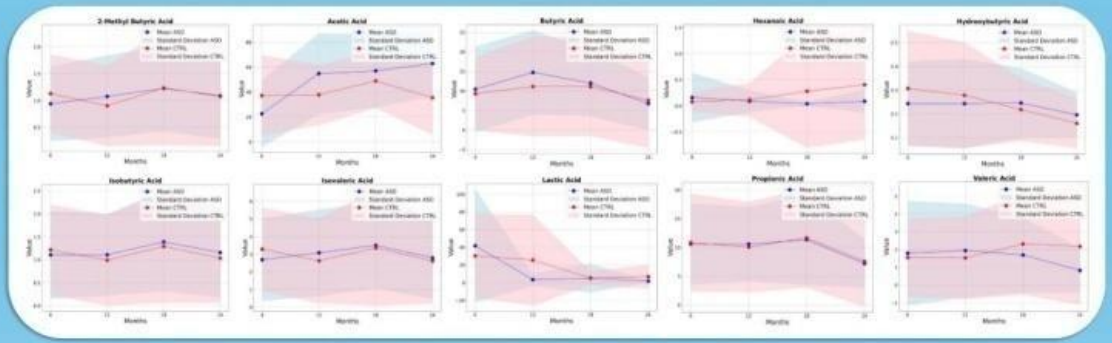


*Figure 4: SCFAs trends over the analyzed time points*

## Conclusion

These findings highlight the necessity of further exploring the interaction between gut microbiota and the onset of ASD. Gaining a thorough understanding of these mechanisms could reveal new insights into the development of ASD and potentially lead to innovative therapeutic strategies in this field.

*Thank you for taking the time to review my poster!*
*Please don't hesitate to inquire about any questions you may have. We welcome the opportunity to exchange ideas and explore potential collaborations!*

* *Correspondence: Alessio Trotta, Theoreo srl, Via degli Ulivi 3, 84090, Montecorvino Pugliano, SA, Italy; trotta@theoreosrl.com*