Metabolomic Profiling in Autism Spectrum Disorder: A Comprehensive Case-Control Study

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1 Introduction

- **Autism Spectrum Disorder** (ASD) affects about 1% of the world's population, with a prevalence of 18 cases per 10,000 individuals. ASD is a term used to define a range of neurodevelopmental disorders denoted by repeated behaviours that begin in childhood and persist into adulthood, causing difficulties in communication and social interaction.
- The **GEMMA** (Genome, Environment, Microbiome and Metabolome in Autism) project was created to describe the relationship between the gut microbiota and the host in order to recognize altered molecular pathways in patients with ASD and identify a metabolomic phenotype of the gastrointestinal microbiota.
- The **aim** of this case-control study is to recognize a distinctive metabolomic pattern of the ASD condition. This may provide an opportunity to understand the altered pathways for early diagnosis, prognosis, and future treatment.

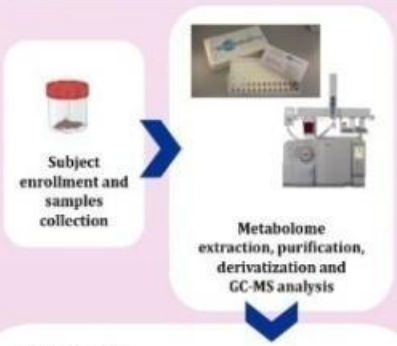
2 Metabolomics Analyses

A total of 72 children's fecal samples were collected from different areas of the world. Metabolomic analyses were conducted on a cohort of 40 patients with ASD (18F,22M), meticulously matched to 32 controls (17F, 15M). In case of mismatches, we applied the adaptive synthetic sampling approach.

Metabolome extraction, purification and derivatization procedures were performed using MetaboPrep GC kit (*Theoreo srl, Montecorvino Pugliano, Italy*), using 2-isopropyl malic acid as internal standard. Derivatization was performed in two steps:

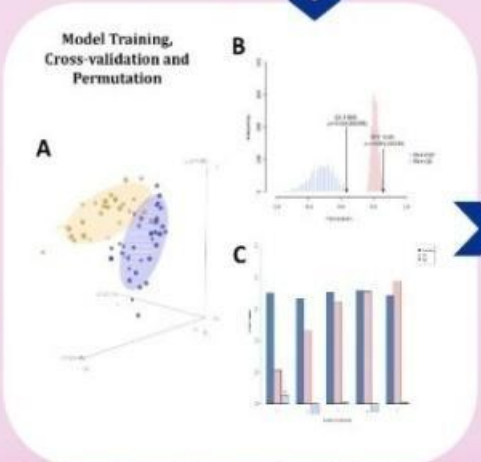
- step 1 → a methoxylamine hydrochloride in pyridine solution was added and vortexed at 1200 rpm for 90 minutes;
- step 2→ 25 µL of a derivatizing solution containing N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA)

Untargeted metabolomic analysis was performed via Gas Chromatography-Mass Spectrometry using a GCMS-2010SE (*Shimadzu Corp., Kyoto, Japan*).

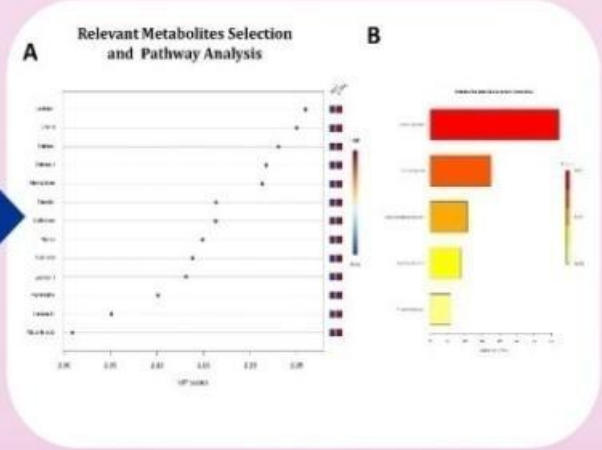


3 Results

- PLS-DA analysis demonstrated good separation between the control and patient groups, with very satisfactory performance as measured by Q^2, R^2 and accuracy. The permutation test indicated a statistically significant p-value confirming the robustness of the model (p-value(Q^2):0.014;p-value (R^2):0.008).
- VIP score plot revealed that the metabolites on which the model showed the highest degree of training are: *lactose, maltitol, maltose, mannobiose, estradiol, cellobiose, ribose, arabinose, asparagine, gluconic acid.*
- Pathway analysis revealed which pathways are most involved, showing significant differences in *lactose degradation* and in *lactose synthesis*.



A. PLS-DA score plot representing the separation of sample healthy controls (yellow) and autistic patients (blue). For each latent variable the percentage of explained variance were reported in brackets. B. Permutation test results based on 2000 iterations. C. PLS-DA classification performance using increasing number of latent variables.



A. Metabolites with VIP score >2 identified in the separation of CTRL and ASD by PLS-DA model. B. Overview of metabolic pathways identified as a function of log10 (p-value).

4 Conclusions

Understanding the composition and function of the gut microbiota in early life is critical.

This long-term study of the fecal metabolome in children with ASD revealed significant changes in gut microbial metabolism and its role in the onset of ASD. The results of this approach help recognize altered metabolic pathways in patients with ASD, identifying a typical metabolic imprinting that paves the way for potential therapeutic interventions targeting the gut microbiota.

Thank you for viewing my poster!
If you're interested in discussing ideas, learning more about our projects, or exploring potential collaborations in this dynamic field, please feel free to contact me. We welcome new perspectives and opportunities for partnership!

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