







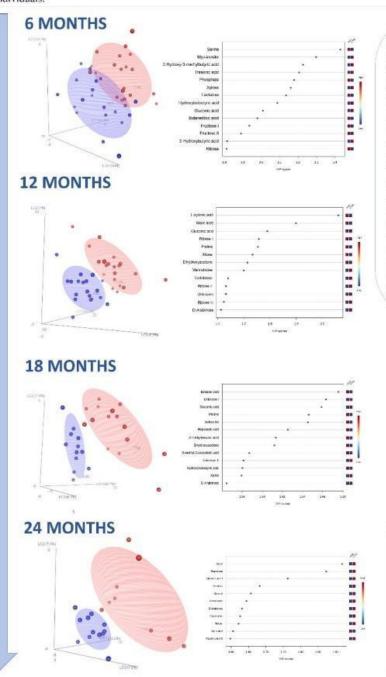


UNRAVELING THE METABOLOMIC LANDSCAPE OF AUTISM SPECTRUM DISORDER: INSIGHTS FROM LONGITUDINAL METABOLOMIC ANALYSIS IN THE GEMMA PROJECT

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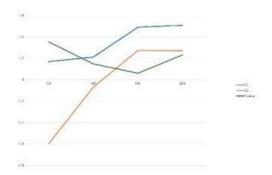
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Introduction: Funded by the European Commission's HORIZON 2020 programme, the GEMMA project investigates Autism Spectrum Disorder (ASD) through a multi-faceted approach encompassing Genome, Environment, Microbiome, and Metabolome. Notably, the risk of developing such a condition increases 10-fold when a child is born in a family where there is already a sibling affected by the same disease. The present study unveils the conclusive findings from longitudinal metabolomic investigations within GEMMA, targeting children at increased risk of developing ASD as siblings of diagnosed individuals.



Methods: Fecal samples were longitudinally collected from a total of 70 individuals (38 ASD; 32 CTRL) at various time points, including 6, 12, 18 and 24 months. Extraction, purification and derivatization were performed using MetaboPrep GC kit (Theoreo srl, Montecorvino Pugliano, Italy) as per manufacturer's instruction. Untargeted analysis performed metabolomic was Chromatography-Mass Spectrometry using a GCMS-2010SE (Shimadzu Corp., Kyoto, Japan). The GC-MS metabolomics results were compiled into a matrix file with comma-separated values, which was then loaded into specialized software (MetaboPredict®, Theoreo srl, Montecorvino Pugliano, Italy) for statistical analysis.

Class separation at the various time points was explored using the Partial Least Square Discriminant Analysis (PLS-DA), that uses multivariate regression techniques to identify linear combinations of the original variables that are predictive of class membership. Moreover, the PLS-DA model was trained to calculate the statistical importance that each metabolite contributes to the overall class separation. These scores are called variable importance in projection (VIP) scores.



Results: The first PLS-DA model evaluated class separation at 6 months, resulting in R2=0.86, Q2 negative, cross-validation accuracy = 0.31, p=0.369. The second model focused on the 12-month time-point and showed R2=0.92, Q2 negative, cross-validation accuracy = 0.61, p=0.182. Similarly, the third model had R2=0.49, Q2=0.36, cross-validation accuracy = 0.74, p=0.057, while the fourth model showed R2=0.51, Q2=0.29, cross-validation accuracy = 0.85, p=0.233. Interestingly, a significant reduction in the p-value emerges at 18 months with a concurrent positive Q2, suggesting that this may be the period when the main differences begin to emerge. Additionally, over time, there is a significant reduction in variability within the ASD group compared to the CTRL group.

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