Investigation of Stool-associated Microbiome of Children with Autism Spectrum Disorder and their Neurotypical Siblings Using Multi-Omics Contrast Analysis

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ABSTRACT: Autism spectrum disorder is a neurodevelopmental condition characterized by social and behavioral impairments. In addition to neurological symptoms, ASD subjects frequently suffer from gastrointestinal abnormalities, thus implying possible links between the gut microbiome and ASD gastrointestinal pathology. The M3 consortium (Metabolic, Microbiome and the Mind) recruited a large cohort of 111 families with one ASD child and one neurotypical (NT) sibling in the same age range to minimize the impact of genetics, diet and environment. Severity of autism in ASD subjects was captured using the Mobile Autism Risk Assessment (MARA). In addition, we collected 365 metadata features across inter- and intra-family variances to permit further investigation of environmental factors’ influences on the ASD microbiome. Gut microbiome from stool samples was characterized at the DNA, RNA and metabolite levels using multi-omics technologies, including 16S V4-HMA region next generation sequencing (16S NGS), 16S V1-V9 RNA PhyloChip® DNA microarray (16S PC), whole-metagenome shotgun sequencing (MTG), metatranscriptomics (MTT) and metabolomics (MTB). Comparing differences in ASD and NT, we found significant differences in compositions of 16S NGS at strain levels (Wald test) as well as higher taxonomical levels (Wilkerson rank sum test) but not by 16S PC or MTG. We also identified specific KEGG or BioCyc functional differences between ASD and NT in MTT and MTG. In addition, we detected bacterial composition and KEGG or BioCyc functional differences associated with severity (MARA score) of the ASD subjects (Spearman’s rank correlation and Wald test). Further integrative analysis revealed specific microbial taxa and KEGG or BioCyc functions that are correlated with one metabolite that was significantly less abundant in the ASD compared to NT subjects. We report here the first comprehensive analysis from the M3 data collection, and demonstrate that multivariate analysis of multi-omics datasets can refine our understanding of the dynamics between microbial metagenome and metabolite profiles and their potential link to gut dysregulation in ASD.

Family-paired M3 cohort minimizing the impact of genetics, diet and environment in multi-omics analysis

Stool samples analysis

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<thead>
<tr>
<th>Microbial Composition</th>
<th>Microbial Gene Expression Profiles</th>
<th>Metabolic Profile</th>
<th>Microbiome Composition</th>
<th>Microbial Gene Expression</th>
<th>Metabonomic Component</th>
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<td>MTG BioCy</td>
<td>MTG BioKeG (function and pathway)</td>
<td>16S PC</td>
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Stool samples analysis

ASD severity correlated with microbial composition and functionality

- Reduced alpha diversity might cause perturbation in the gut microbiome of the subject and accentuate the symptoms of ASD.
- Metabolite A (reaction A) is depleted in the most severe ASD cases. The Metabolite associated to reaction A has been reported as an important molecule against dysbiosis.
- Reduced abundance of Akkermansiaaceae family is associated with ASD severity. This could indicate a thinner GI mucous barrier in children with severe ASD compared to others. The result might reflect an indirect evidence of impaired gut permeability in children with severe ASD (L. Wang et al. 2011).

Significant differences in bacterial and metabolite composition between ASD and NT might underlie gastro-intestinal and neurodevelopmental symptoms in ASD

**Conclusion and future work**

- Using multi-omics techniques to discover several differences between the ASD subjects and their NT siblings. This difference areas visible among the microbial community, the functional annotation of their genome and transcriptome and the metabolite profiles. The severity of ASD is correlated to the microbiome.
- The integration of these data sets through a network analysis permitted a better understanding of the complex interaction between the different species and their influence over the production of essential metabolites.
- These connections need to be further confirmed by in vivo and/or in vivo assays, in order to develop treatment in the future.

**Acknowledgement & references**

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