Analysis of intestinal microbiota of children with autism spectrum disorders

Abstract

The aim of this doctoral thesis was to conduct a detailed analysis and demonstrate differences in the composition of the gut microbiota, based on the analysis of stool samples, in three study groups: children with autism spectrum disorders (ASD, *n* = 71), their neurotypical siblings (*n* = 29), and a control group of unrelated neurotypical children (*n* = 44), in the context of diet analysis, short-chain fatty acids (SCFA), and gastrointestinal symptoms.

High-throughput next-generation sequencing (NGS) of the hypervariable regions V3–V4 and V7–V9 of the *16S rRNA* was performed to determine the microbiota composition. Bioinformatic analysis was conducted using the Qiime2 environment with additional plugins. The Sidle version of the Short MUltiple Reads Framework (SMURF) algorithm was used to conduct simultaneous analysis based on two sequenced amplicons. Surveys were conducted to determine important covariates that could influence the gut microbiome. The survey assessed the scale of gastrointestinal symptoms, infant feeding, as well as diet in the 7 days preceding the collection of stool samples for microbiota study. HPLC analysis was performed to assess SCFAs in stool samples.

The study demonstrated significant differences in the gut microbiota composition between groups, indicating increased diversity and evenness in the microbiota of neurotypical siblings compared to children with ASD and the unrelated neurotypical control group. A total of 53 bacterial taxa with abundances different between the study groups were identified, with increased abundance of taxa belonging to the family Lachnospiraceae, genus *Gelria* and *Desulfovibrio* in ASD group compared to unrelated controls, and decreased abundance of the genera *Faecalitalea*, *Parabacteroides*, and *Odoribacter*.

We have confirmed the literature reports that children with autism spectrum disorders experience gastrointestinal symptoms more frequently compared to unaffected children. Significant differences in the dietary habits of children with ASD were pointed out, finding differences also during infancy. No significant differences in SCFA were found.

The results provide information about differences in gut microbiota of children with ASD compared to their neurotypical siblings and unrelated neurotypical children in the Polish population and provide a good basis for further functional analysis of microbiomes in this type of neurodevelopmental disorder. The use of a sequencing approach of two amplicons containing more than one hypervariable region for taxonomic identification significantly improves the resolution of the analysis, increasing the accuracy of taxonomic identification, which constitutes a significant advancement in microbiome research and a substantial contribution to this field of research.