BITS :: Call for Abstracts 2021 - Poster

Type	Poster
Session	Multiomics and Single Cell Analysis
Title	Human – microbiota crosstalk in Autism Spectrum Disorder, a pilot study.
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Motivation

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by communication impairments, limited social interaction, restricted interests, repetitive behaviors and stereotypies [1,2]. It manifests within the first 3 years of age and lasts for a lifetime with dramatic personal, familial, social and economic consequences. ASD affects much more males than females (male:female=5:1) and its prevalence, that is continuously increasing, interests 2.24% of children and 1% of the general population [1,3]. Although genetics play a key role in ASD, its etiology is complex and uncertain. Since most of individuals with ASD suffer from comorbidities including gastrointestinal disorders, increased intestinal permeability, inflammation and allergies, a gene-environment interaction has been proposed as ASD triggering factor [3].
Gut dysbiosis has been frequently associated with many neurological disorders including ASD [4]. Thanks to the last advances in metagenomics, much progress has been made in the knowledge of gut microbiota profile and its possible role in healthy and diseases was proposed. To date, human gut metagenomic studies have been focused on the prokaryotic microorganisms, while the eukaryotic profile is poorly explored, especially in ASD. Interestingly, a recent study detected miRNAs, secreted by intestinal epithelial cells, in a human stool sample. It also demonstrated that microRNAs can infiltrate gut bacteria, regulate their gene transcription and, consequently, their growth thus conceiving a cross-talk between human intestine and bacteria [5].
Here, we propose a pilot study to define the prokaryotic, eukaryotic and smallRNA profile of faeces collected from children with ASD and neurotypical controls. The aim was to find, in ASD, a possible pathological cross talk between epithelial microRNAs and gut microorganisms. Moreover, our ultimate goal was to identify miRNA and/or microbial markers useful for patient stratification and personalized treatments.

Methods

We isolated DNA and total RNA from stools collected from 6 children with ASD (5 males and 1 female) and 6 neurotypical controls matching for age and sex. SmallRNA library was generated from total RNA and sequenced on Illumina NextSeq500and performed paired-end sequencing, reaching about 30mln per sample. Both 16S and 18S were amplified for each DNA and Illumina libraries prepared. NGS was performed by Illumina MiSeq platform coupled with Flowcell V3 and forward and reverse reading, reaching about 22Milion of sequences.
SmallRNA reads were mapped with Bowtie against ncRNAdb (an in-house-developed reference database representing a comprehensive and non-redundant dataset of public ncRNA sequences and annotations). An evident heterogeneity in the expressions of several individual references required an accurate management of the expression normalization step, that was done by applying a reference-free clustering of the sequences with SEED [6] and by computing the scaling factors of TMM normalization on the cardinalities of the clusters. Expression data were analyzed with edgeR.
Metatassonomic analysis were performed in R (4.0.3) employing Dada2 pipeline, against DADA2-formatted reference databases latest available version, that is Silva v138 for 16S and Siva v132 for 18S.

Results

As for smallRNA analysis, only miRNA class results was considered: no statistically significant expression differences was found between ASD and control groups nor a common trend because of a great samples heterogeneity. Several differentially expressed hsa-miRNAs can be identified among ASD samples, and none is common for all ASD samples. Taking into account that multiple miRNAs target the same gene and single miRNA target multiple genes, we are evaluating the miRNA-target gene network involving the identified miRNAs.
Metatassonomic analysis returns heterogeneous results among samples, but differences among ASD and controls in bacterial Family composition allow to identify 8 Families exclusively observed in ASD and 7 in

controls. Furthermore, ASD samples display a reduced variability in Genus compared to controls. Different Family composition was observed also for 18S analysis; the ASD group includes 9 exclusive eukaryotes Families, while the control group 5. Vegetables Families were excluded from the results. We will proceed evaluating cross-reactions among the microbial Families identified within the same sample, and their possible role in the growth regulation of other microbial species.
This pilot study revealed the advantage of a multiomics approach in defining the microbial and miRNAome profile to identify a possible interaction between human miRNAs and gut microbiota, through the modulation of microbial gene expression.

Info

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Figure

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Availability -

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