BITS :: Call for Abstracts 2022 - Oral communication

Type	Oral communication
Session	Multi-omics data analysis and integration
Title	Host–gut microbiota cross-talk in autism via microbiota, mycobiota and small ncRNAs
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Motivation

Intestinal microorganisms impact health by maintaining gut homeostasis and shaping the host immunity, while gut dysbiosis associates with many conditions including autism, a complex neurodevelopmental disorder with multifactorial aetiology. In autism, gut dysbiosis correlates with symptom severity and is characterised by a reduced bacterial variability and a diminished beneficial commensal relationship. Microbiota can influence the expression of host microRNAs that, in turn, regulate the growth of intestinal bacteria by means of bidirectional host-gut microbiota cross-talk.
In this scenario, it emerges that microbes and small non-coding RNA (sncRNA) of faecal samples should be considered and studied as a whole to comprehend how microbial strains interact among each other and with the host. In this pilot study, we defined through “omics” technologies, the faecal microbiota and mycobiota profile as well as the sncRNA profile (particularly miRNAs and piRNAs) profile of a small group of individuals with ASD and neurotypical controls. Our aim was to find markers of ASD among stool microbial and transcriptional modulators for the possible relationship between them and the host.

Methods

We isolated DNA and total RNA from stools collected from 6 children with ASD (5 males and 1 female) and 6 neurotypical matching individuals. SmallRNA library was generated from total RNA and sequenced by Illumina NextSeq500; paired-end sequencing was performed reaching 30mln reads/sample. 16S and 18S rDNA were amplified by PCR and Illumina libraries prepared. Paired end sequencing was performed by Illumina MiSeq Flowcell V3, 2X300bp, returning an average of 0.8mln reads/sample for 16S and 28mln reads/sample for 18S.
SmallRNA reads were mapped using Bowtie aligner, against Arena-Idb [1]. 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 and by computing the scaling factors of TMM normalization on the cardinalities of the clusters. Expression data were analyzed with edgeR. The BH multiplicity correction method was used to control the FDR.
Metataxonomic analysis were performed in R (4.0.3) employing Dada2 pipeline, against Silva v138 db for 16S and Siva v132 db for 18S. To evaluate statistically significant differences between ASD and controls at genus level, the univariate DESeq2 method was used. Default Wald test was applied in DESeq2, and significance threshold was set to p-value < 0.05 for the 16S analysis, while for the 18S analysis all results were considered.
All identified miRNA and piRNA were annotated based on miRPathDB [2] and piRNAdb [3]. Gene targets common to miRNAs and/or piRNAs from different samples were studied. Gene targets were annotated using KEGG and MSigDB-Hallmark gene sets [4] using KEGGREST [5] and R function msigdbr (v7.4.1) [6].

Results

Due to high heterogeneity of the sample compositions, we performed the metataxonomic and the sncRNA analysis comparing each ASD sample to the whole control set. Based on our and literature results, we try to disentangle the complex cross-talk between the dysregulated gut microbial strains and up-regulated sncRNAs in ASD. We confirmed the reduction of bacteria involved in healthy gut maintenance in ASD, such as families involved in SCFAs production, highlighting the possible role of dysregulated microbiome metabolites in ASD aetiology. In ASD, only the Debaryomycetace fungi family was predominant, consisting of Candida-Lodderomyces_clade and Meyerozyma-Candida_clade. Candida overgrown produces root-like structures that penetrate the intestinal wall, causing the leaky-gut syndrome, already detected in ASD, and allowing toxins and food antigens to enter the bloodstream.
We profiled miRNAs and piRNAs and performed the target pathway analysis of the dysregulated ones. The most represented miRNAs in all ASD stools were hsa-miR-2110 and hsa-miR-657. The increased expression of the latter, is associated with inflammatory response in gestational diabetes mellitus, a known risk factor for autism in offspring.
The up-regulated miRNAs and piRNAs negatively influence 26 genes belonging to pathways that are associated with ASD or its comorbidities, such as in intestinal permeability and inflammation.
Here, we propose a novel approach to analyse faeces as a whole, and for the first time, we found up-regulated miRNAs and piRNAs in faecal samples of patients with autism [7]. Results need to be confirmed in a larger cohort.

Info

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2- https://mpd.bioinf.uni-sb.de/
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4- https://www.gsea-msigdb.org/gsea/msigdb/
5- https://bioconductor.org/packages/release/bioc/html/KEGGREST.html
6- https://igordot.github.io/msigdbr/
7- Chiappori, F.; Cupaioli, F.A.; Consiglio, A.; Di Nanni, N.; Mosca, E.; Licciulli, V.F.; Mezzelani, A. Analysis of Faecal Microbiota and Small ncRNAs in Autism: Detection of miRNAs and piRNAs with Possible Implications in Host–Gut Microbiota Cross-Talk. Nutrients 2022, 14, 1340. https://doi.org/10.3390/nu14071340

Acknowledgements: EU project GEMMA (grant agreement No 825033) https://www.gemma-project.eu/it/

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Submitted on	29.04.2022