Host-microbiome cross-talk: analysis of non-coding RNAs in stools of children with autism and dysbiosis

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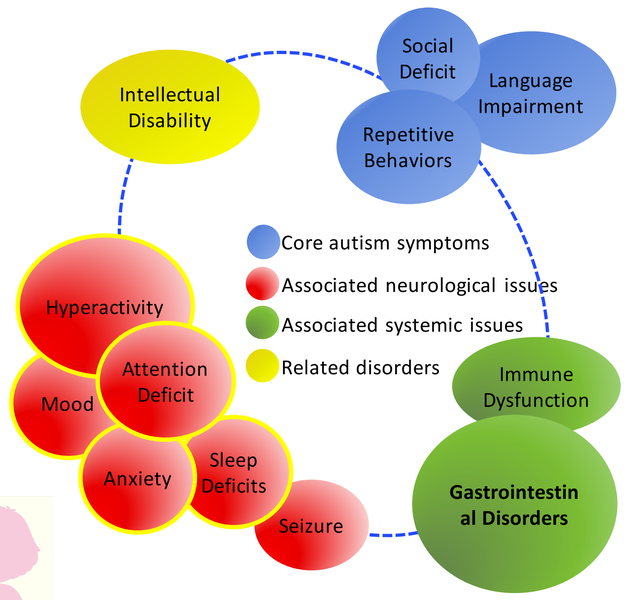
Introduction

Autism spectrum disorders (ASD) are a complex neurodevelopmental condition, characterized by deficit in social interaction and communication, often accompanied by gastrointestinal disorders and dysbiosis. ASD aetiology is attributed to the effect of environmental factors in genetically predisposed individuals. Intestinal microorganisms represent interesting environmental candidates since specific alterations in gut microbial profile are linked to the severity of ASD symptoms (1). It has been demonstrated that gut microbiota can influence the expression of host miRNAs, a class of small non-coding RNAs (ncRNAs) that post-transcriptionally regulate gene expression by binding target mRNAs; while host miRNAs, secreted by intestinal epithelial cells, can infiltrate gut bacteria regulating their transcription and growth, thus highlighting a gut-bacteria bidirectional cross-talk (2). We previously analysed the microbiota and small ncRNAs profiles in faeces of children with ASD and neuro-typical controls, highlighting possible cross-talk (3). Now, we improve this study investigating also long ncRNAs in faeces.



Aim

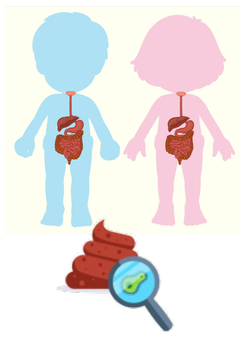
Investigate the possible role of ncRNAs in host-microbiome bidirectional cross-talk in autism.

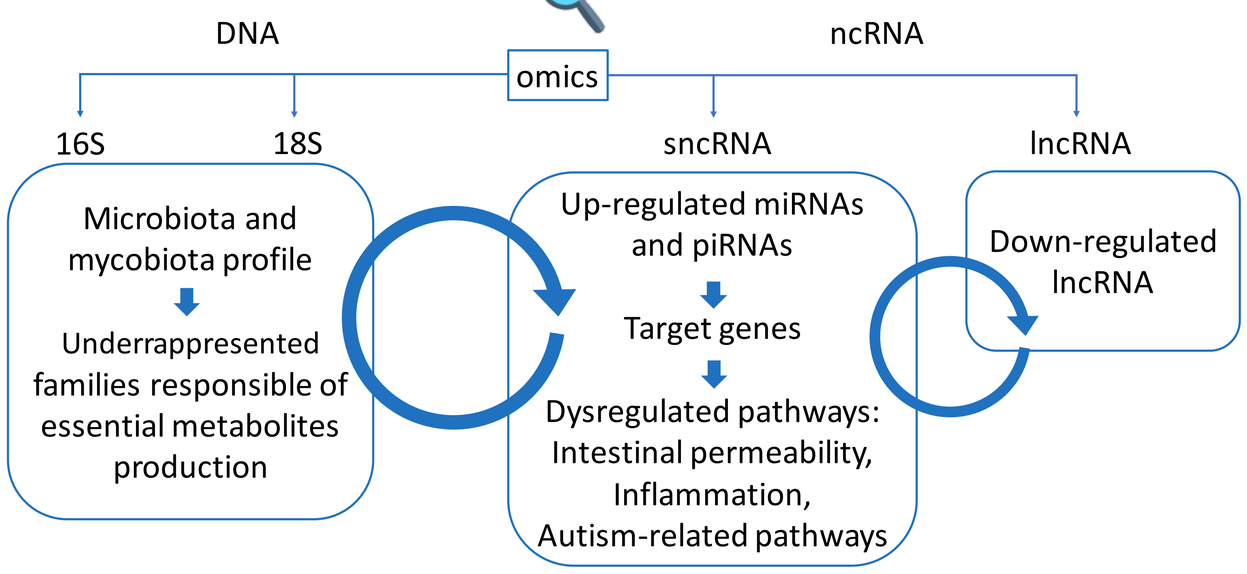
Method

-Isolation of DNA and total RNA, including small-RNA, from faeces of children with autism and neurotypical controls

-Omics analysis

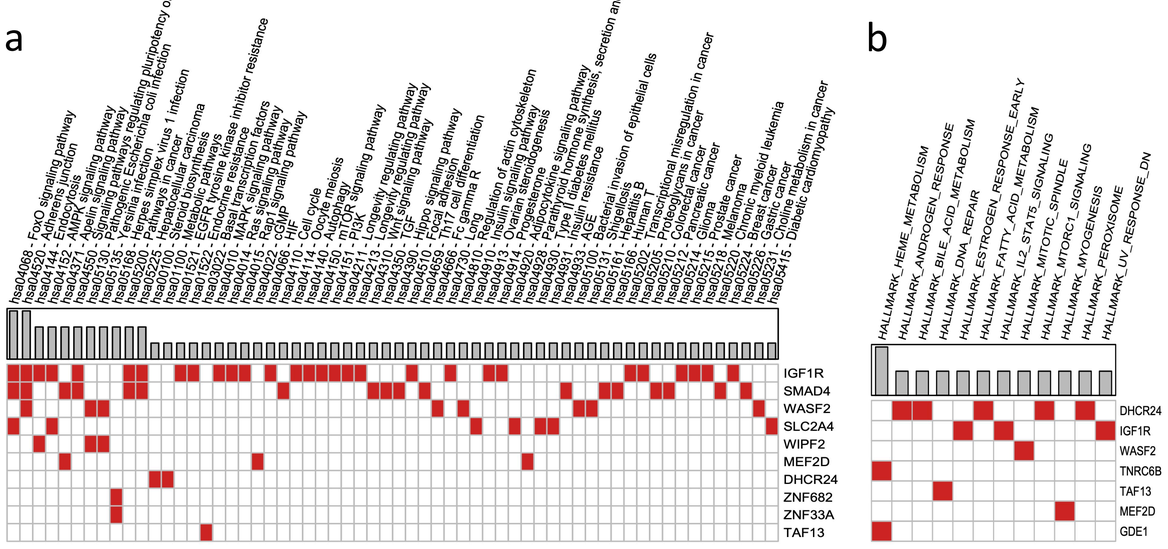
- metatassonomic: 16S and 18S sequencing
- small-RNAseq
- set up of new bioinformatics approach to investigate the profile of long ncRNAs from small-RNAseq





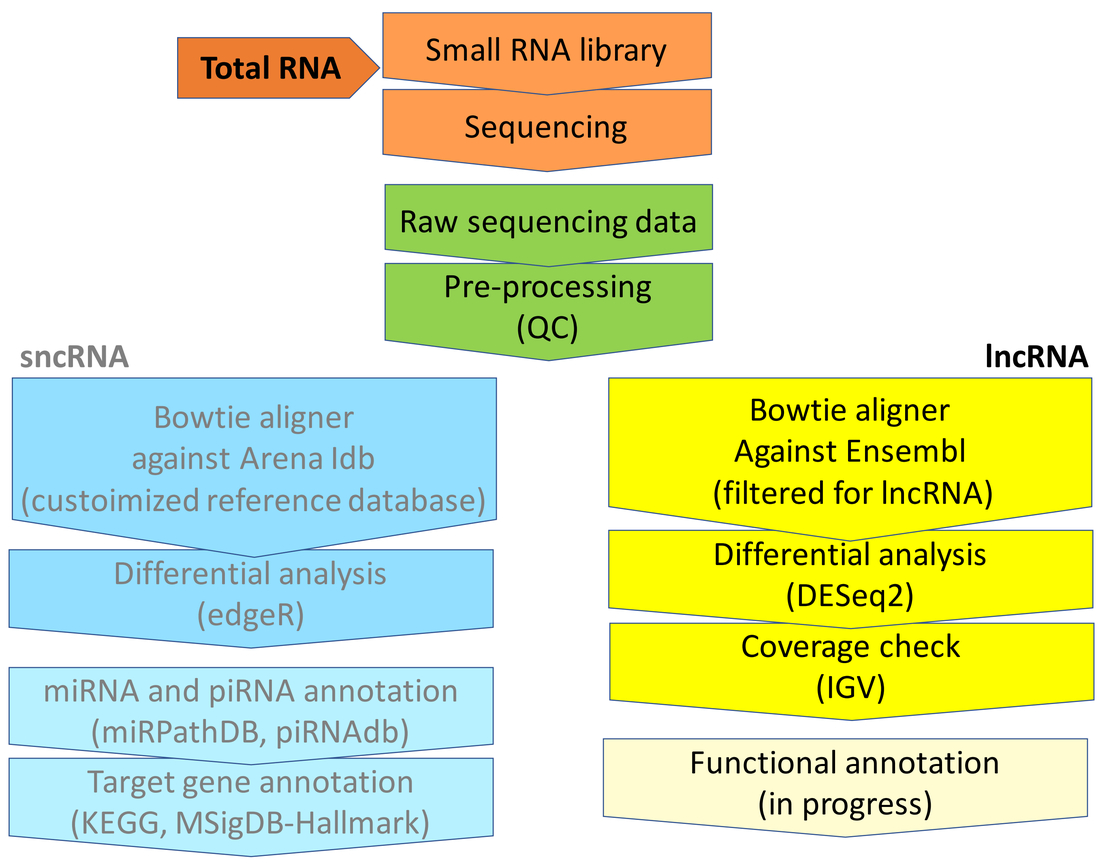
Results (I)

Functional annotation of miRNA and piRNA target genes from KEGG (**a**) and MSigDB-Hallmark (**b**) highlights the involvement of cell–cell junctions, bacterial invasion, inflammation and metabolite signalling processes. Interestingly, these are known to be linked to autism.



Results (II)

New pipeline to investigate lncRNAs from smallRNAseq



Results (III)

ncRNAs annotation and analysis highlighted miRNAs and piRNAs common genes target, as well as lncRNA interactions with transcription factors, proteins and histone modifications.

Significative		Common interactions
miRNA	42 up-regulated (3/6 samples)	Target genes: *TTN (already associated with ASD), N4BP1*, *SLC2A4*, *SLC12A6* and *ZNF33A*
piRNA	84 up-regulated (4/6 samples)	
lncRNA	30 down-regulated (3/6 samples)	Transcription factors: *AR, CTCF, MYC, RELA* (all already linked to ASD) and *FOXA1, ESR1, GATA6, POU5F1, TAL1* Proteins: POLR2A. Missense variants of *POLR2A* gene are described in ASD Histone modification: *H3K4me1, H3K4me2, H3K4me3* (all already linked to ASD) and *H3K27ac, H3K27me3, H3K36me3, H3K9ac*

Discussion

This is the first study that analyses ncRNAs in stools by small RNA-seq: classic small RNA-seq allows the profiling of dysregulated miRNAs and piRNAs (this last, detected for the first time in faeces), while, aligning small reads against lncRNA genes, represents a pioneering approach to investigate long ncRNAs, supplying the degradation of nucleic acids in faeces. The preliminary results obtained are promising, however need to be experimentally confirmed, related to the detected miRNAs and functionally annotated. This underlines the need to develop an *ad hoc* pipeline that exploits short reads to map long ncRNAs.

Stool ncRNAs analysis can help in disentangling the host-gut microbiota cross-talk for deciphering dysbiosis molecular mechanisms.

References

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