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Pilot Study: Is Autism Spectrum Disorder Related with Formula?

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Introduction. Autism Spectrum Disorder (ASD), characterized by social communication deficits and repetitive behaviors, may have a genetic link [1, 2]. Key factors affecting neurological development include breastfeeding and the baby’s social environment [3, 4]. Additionally, the gut microbiome, often differentiated in those with ASD who also experience intestinal issues, is a potential contributing factor to ASD development [5].

Objectives. We will employ machine learning-based feature selection algorithms to detect taxonomic differences between breast milk and formula. Subsequently, we will investigate whether these identified taxa exhibit any relationship with Autism Spectrum Disorder (ASD) by examining two relevant datasets.

Methods.

Data. Table 1 details three datasets used to study taxa differences between breast milk and formula, and two ASD-related datasets to explore potential links between these taxa and ASD. All datasets were downloaded from NCBI repository.

Table 1: Characteristics of the selected datasets

Accession Number	Experiment	Samples	Labels	Taxa (features)
PRJNA633365 [6]	breast milk - formula	65	22 - breastfeed 43 - formula-feed	1,202
DRA007599 [7]	breast milk - formula	60	24 - breastfeed 36 - formula-feed	2,521
PRJNA515307 [8]	breast milk - formula	40	20 - breastfeed 20 - formula-feed	1,779
PRJNA578223 [9]	ASD	96	48 - healthy-control 48 - ASD	18,749
PRJNA624252 [10]	ASD	50	20 - healthy-control 30 - ASD	3,249

Feature Selection. We applied the methodology described in [11] which combines a DADA2-based script and the Recursive Feature Selection (REFS) algorithm designed for biomarker discovery and consists of four phases:

- dataset selection criteria
- raw data processing
- feature selection
- testing

The methodology was applied to identify taxa differences between breast milk and formula using three independent datasets: one as a discovery dataset (PRJNA633365) and the remaining two for testing (DRA007599 and PRJNA515307). For the ASD experiments, we used the same methodology applied to two datasets (PRJNA578223 and PRJNA624252) related to ASD, but in this case, we replaced the feature selection phase with the taxa resulting from the previous experiment, Fig. 1.

Results. After applying the methodology to the three datasets related to breast milk and formula, nine bacteria were identified in the discovery dataset PRJNA633365, at genus level: *Cutibacterium*, *Lactobacillus*, *Bacteroides*, *Bifidobacterium*, *Bifidobacterium*, *Lactobacillus*, *Bilophila*, and *Romboutsia*; at order level: *Enterobacteriales*. Five out of nine and three out of nine were found in the testing datasets DRA007599 and PRJNA515307 respectively.

In all cases, the algorithm with the best performance after the testing phase was KNeighbors with AUCs of 0.77 for PRJNA633365, 0.63 for DRA007599 and 0.75 for PRJNA515307. In the ASD experiments, we found two out of nine for PRJNA578223 and tree out of nine for PRJNA624252. For both cases, the algorithm with the best performance was the Multi-layer Perceptron with AUCs of 0.67 for PRJNA578223 and 0.62 for PRJNA624252. The bacteria found in the ASD datasets are, at genus level, *Bacteroides* and *Bilophila* in both datasets and *Lactobacillus* only in PRJNA624252. The literature shows *Bacteroides* and *Lactobacillus* are increased [12, 13] in people diagnosed with ASD, in contrast with *Bilophila* which is decreased [13].

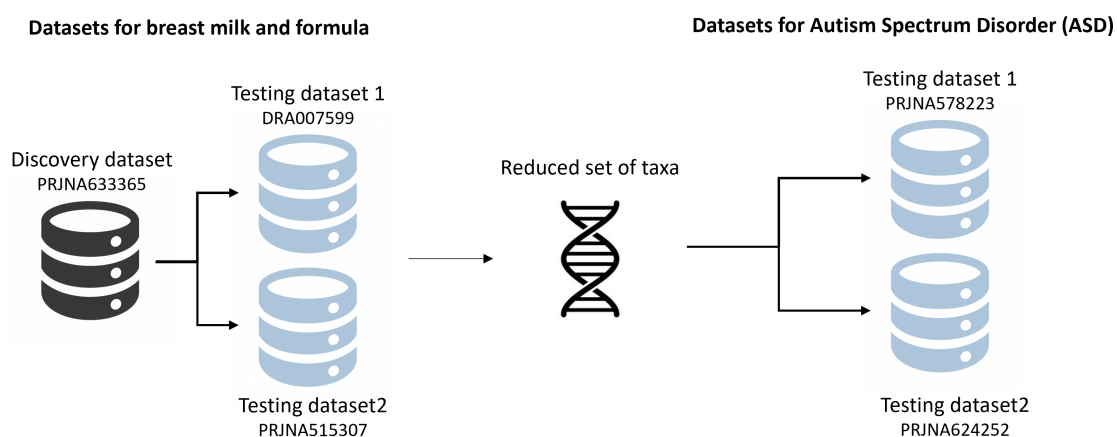


Figure 1: General overview of the experiments in this study using the methodology described in [11].

Conclusion. We applied a reproducible methodology that makes use of machine learning methods to identify taxa differences between breast milk and formula. These results were used to study a possible relationship between these taxa and ASD. The results obtained in this pilot study are promising. While further research with more experimentation is necessary, this study provides the first steps to explain the roles that breast milk and/or formula play in the development of ASD and other conditions.

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