Functional survey of the pediatric multiple sclerosis microbiome

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INTRODUCTION & PURPOSE

We examined the gut microbiome functional potential by metagenomic analysis of stool samples from pediatric multiple sclerosis (MS) cases and matched controls using a case-control design.

METHODS

Persons ≤21 years old enrolled in a Canadian Pediatric Demyelinating Disease Network study

All MS cases met McDonald criteria, had symptom onset <18 years of age and had either no prior disease-modifying drug (DMD) exposure or were exposed to beta-interferon or glatiramer acetate only

20 MS cases were matched to 20 unaffected controls by:

- Sex, age at stool sample collection (± 3 years), stool consistency (Bristol Stool Scale) and, when possible, by race

Shotgun metagenomic reads were generated using the Illumina NextSeq platform.

We compared:

1. Functional diversity of enzymes (Enzyme Commission numbers) and proteins (KEGG orthology) using Wilcoxon Rank Sum test
2. Metabolic pathways (MetaCyc) relative abundance and prevalence
   - By disease status (MS case or unaffected control)
   - By disease-modifying drug exposure (DMD *naïve* vs. *ever* exposed MS case vs. unaffected control)
3. Gene ontologies (GOs) relative abundance and prevalence by disease status

Differential abundance analyses was assessed using ALDEx2 (Wilcoxon Rank Sum test).

Differential proportional analyses (prevalence) was assessed using the Fisher Exact test

RESULTS

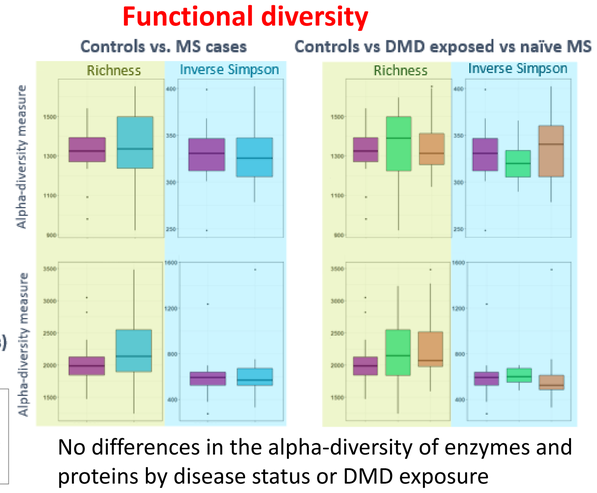
Characteristics of the pediatric-onset multiple sclerosis cases and unaffected controls

Characteristic	MS cases, n=20	Controls, n=20
Female, n (%)	16 (80%)	16 (80%)
Age at stool sample collection, years: mean (SD)	16 (4)	15 (3)
Age at symptom onset, years: mean (SD)	13 (4)	N/A
Race: white, n (%)	11 (55%)	9 (45%)
Body Mass Index: mean (SD)	23 (5)	21 (4)
Hard stool (Bristol stool scale, types 1-2), n (%)	4 (20%)	4 (20%)
Dietary metrics (Block Kids Screener): mean (SD)		
Energy (kcal/day)	950 (337)	1151 (535)
Fibre (g/day)	9 (5)	12 (7)

All cohort characteristics were similar between MS cases and controls (p>0.05, Wilcoxon test)

DMD use for the MS cases (pre-stool sample)	
Naïve	8 (40%)
Exposed	12 (60%)
Beta-interferon	*7 (35%)*
Glatiramer acetate	*5 (25%)*

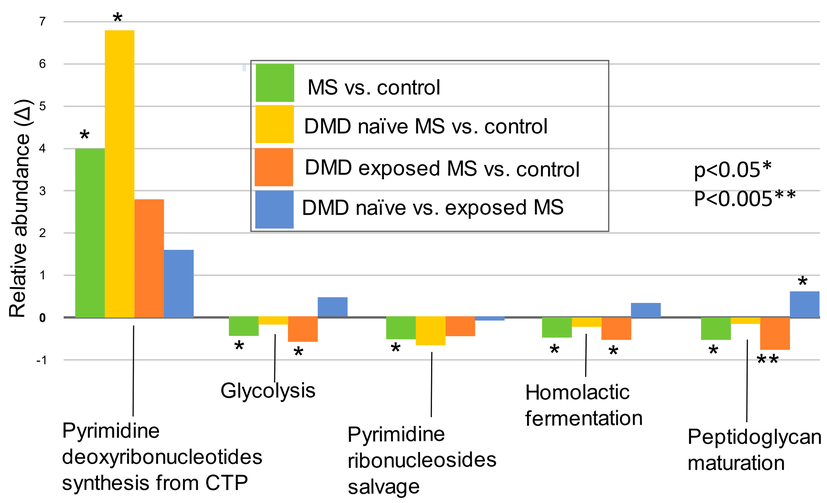
Functional diversity



No differences in the alpha-diversity of enzymes and proteins by disease status or DMD exposure

RESULTS (CONTINUED)

Metabolic Pathways - *Relative Abundance*

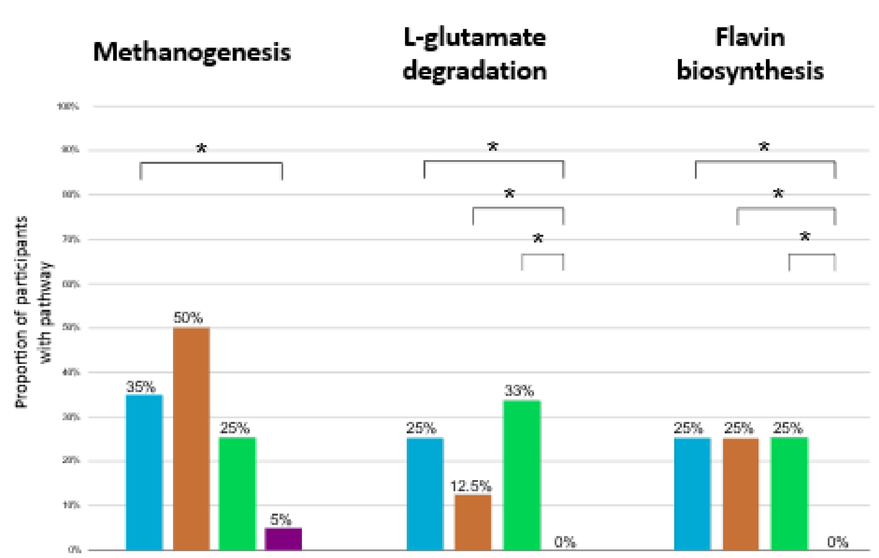


Homolactic fermentation: lactic acid production; associated with anti-inflammatory effects[1].

Peptidoglycan maturation: components of the bacterial capsule, found in nearly all bacteria, circulate throughout human body and are necessary for proper immune cell homeostasis[2]. ***Higher*** *for MS DMD naïve vs exposed*

Both pathways: ***Lower*** *for MS cases vs controls,* ***Lower*** *for MS DMD exposed vs controls*

Metabolic Pathways - *Prevalence*

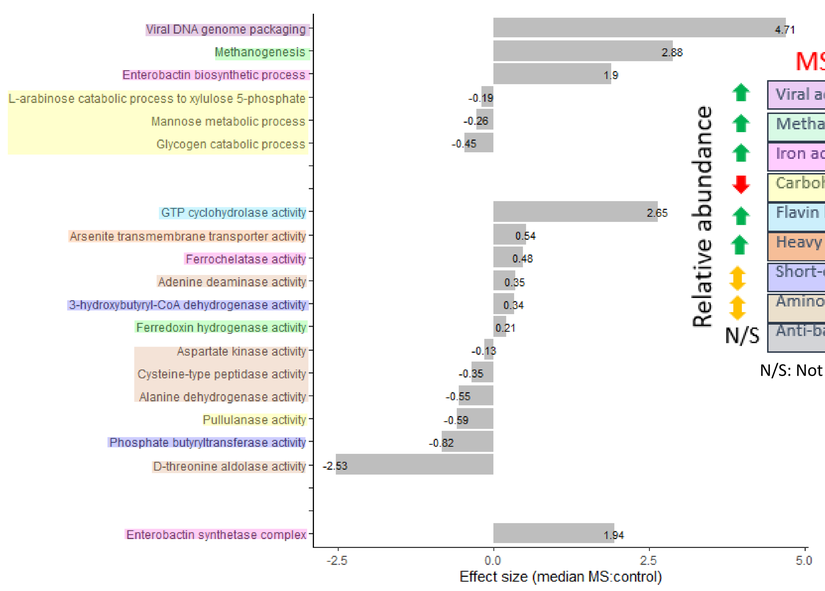


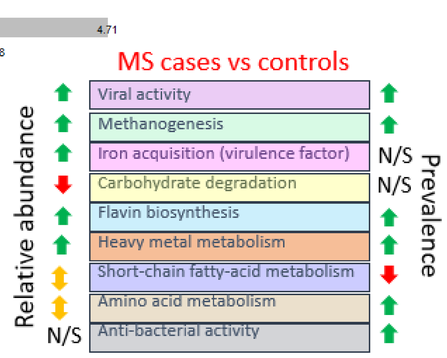
MS cases were enriched (vs controls) for:

- **Methanogenesis:** Methane production by *Archaea*
- **L-glutamate degradation:** Produces propionate, a short-chain fatty acid, from amino acid glutamate
- **Flavin biosynthesis (archaea):** Produces riboflavin (vitamin B2)

Differences were not significant for the DMD naïve vs. exposed MS cases, p>0.05

Gene Ontology - *Relative Abundance*

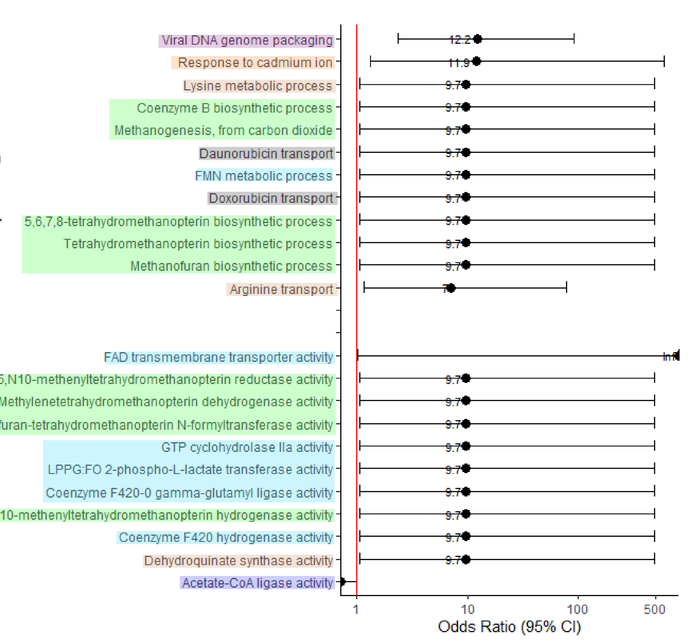




N/S: Not significant

Relative abundance for the MS cases vs. controls (Δ)

Gene Ontology - *Prevalence*



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CONCLUSION

No overall difference in functional diversity for the pediatric onset MS cases vs controls. However, relative to controls, the MS cases exhibited:

Higher
- Archaea-related methanogenesis
- Archaea-related Vitamin B2 (riboflavin) production
- Viral activity
- Iron acquisition via enterobactin
- Heavy metal activity

Lower
- L-glutamate degradation (to propionate; a short-chain fatty acid)
- Peptidoglycan maturation
- Homolactic fermentation (lactate production)
- Carbohydrate degradation
- Glycolysis

Peptidoglycan maturation differed by disease-modifying drug status; for example was higher for the DMD naïve vs exposed MS cases.

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