Functional survey of the pediatric multiple sclerosis microbiome

A. Mirza1, F. Zhu1, N. Knox2, J. Forbes3, G. van Domselaar2, CN. Bernstein4, M. Graham2, R.A. Marrie5, J. Hart5, E.A. Yeh3, A. Bar-Or6, J. O’Mahony7, W. Hsiao1, B. Banwell8, E. Waubant3, H. Tremlett1

1University of British Columbia, Vancouver, BC Canada, 2Public Health Agency of Canada, Winnipeg, MB Canada, 3University of Toronto, Toronto, ON Canada, 4University of Manitoba, Winnipeg, MB Canada, 5University of California, San Francisco, San Francisco, CA USA, 6University of Pennsylvania, Philadelphia, PA USA, 7The Hospital for Sick Children, Toronto, ON Canada, 8Children’s Hospital of Philadelphia, Philadelphia, PA USA

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 were assessed using ALDEx2 (Wilcoxon Rank Sum test).

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

RESULTS (CONTINUED)

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MS cases, n=20</th>
<th>Controls, n=20</th>
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</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>16 (80%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>Age at stool sample collection, years: mean (SD)</td>
<td>16 (4)</td>
<td>15 (3)</td>
</tr>
<tr>
<td>Age at symptom onset, years: mean (SD)</td>
<td>13 (4)</td>
<td>N/A</td>
</tr>
<tr>
<td>Race: white, n (%)</td>
<td>11 (55%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Body Mass Index: mean (SD)</td>
<td>23 (5)</td>
<td>21 (4)</td>
</tr>
<tr>
<td>Hard stool (Bristol stool scale, types 1-4), n (%)</td>
<td>4 (20%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Dietary metrics (Block Kids Screener): mean (SD)</td>
<td>950 (337)</td>
<td>1151 (535)</td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
<td>95 (5)</td>
<td>12 (7)</td>
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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%)

REFERENCES


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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.