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Gut microbiota composition and relapse risk in pediatric MS: a pilot study

Helen Tremlett[1], **Douglas W. Fadrosh**[2], **Ali A. Faruqi**[2], **Janace Hart**[2], **Shelly Roalstad**[3], **Jennifer Graves**[2], **Susan Lynch**[2], and **Emmanuelle Waubant**[2] **on behalf of the US Network of Pediatric MS Centers**

[1]University of British Columbia, Vancouver, BC, Canada

[2]University of California, San Francisco, San Francisco, CA, United States

Corresponding author: Professor Helen Tremlett, Faculty of Medicine (Neurology), University of British Columbia, Room S178, 2211 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada. Tel: 1-604- 822-0759. helen.tremlett@ubc.ca.

Statistical analyses were performed by Helen Tremlett (University of British Columbia)

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The US Network of Pediatric MS Centers (authors listed in alphabetical order): Greg Aaen[1], Anita Belman[2], Leslie Benson[3],, Charlie Casper[4], Tanuja Chitnis[3], Mark Gorman[3], Yolanda Harris[7], Lauren Krupp[2], Tim E Lotze[6], Sabina Lulu[7], Jayne Ness[5], Cody Olsen[4], Erik Roan[4], Moses Rodriguez[5], John Rose[4], Timothy C Simmons[4], Jan-Mendelt Tillema[5], Wendy Weber[4], Bianca Weinstock-Guttman[9]

1. Loma Linda University, Loma Linda, CA, United States; 2. Stony Brook University, Stony Brook, NY, United States; 3. Harvard University, Cambridge, MA, United States; 4. University of Utah, Salt Lake City, UT, United States; 5. Mayo Clinic, Rochester, MN, United States; 6. Baylor College of Medicine, Houston, TX, United States; 7. University of California, San Francisco, San Francisco, CA, United States; 8. University of Alabama, Birmingham, AL, United States; 9. State University of New York at Buffalo, Buffalo, NY, United States

[3]University of Utah, Salt Lake City, UT, United States

Abstract

We explored the association between baseline gut microbiota (16S rRNA biomarker sequencing of stool samples) in 17 relapsing-remitting pediatric MS cases and risk of relapse over a mean 19.8 months follow-up. From the Kaplan-Meier curve, 25% relapsed within an estimated 166 days from baseline. A shorter time to relapse was associated with *Fusobacteri*a depletion (p=0.001 log-rank test), expansion of the *Firmicutes* (p=0.003), and presence of the Archaea *Euryarchaeota* (p=0.037). After covariate adjustments for age and immunomodulatory drug exposure, only absence (vs presence) of *Fusobacteria* was associated with relapse risk (hazard ratio=3.2 (95%CI: 1.2-9.0), p=0.024). Further investigation is warranted. Findings could offer new targets to alter the MS disease course.

Keywords

pediatric multiple sclerosis; gut microbiota; 16S rRNA; relapse risk; survival analyses; Kaplan-Meier; Cox regression

Background

Gut microbiota perturbations have been associated with disease activity in animal models of MS[1,2,3] but the association with activity in MS subjects is unknown. In animal models representing relapsing-remitting MS, for instance, a germ-free environment has been associated with a milder disease course.[1,2] In addition, oral administration of members of the *Bacteroides* phylum (*Bacteroides Fragilis*) have been associated with a lower 'clinical' score in relapsing models of MS.[3,4] Currently, relatively little is known as to what might trigger or facilitate the onset of a new MS relapse. Environmental exposures such as stress, season, sunlight, vitamin D and recent viral infections have been linked to risk of relapse, with the presumed pathway(s) being through immune system modulation. Interestingly, these environmental factors also influence the gut microbiota which likewise modulates the immune system which is known to be affected in MS.[1,2,3] Further, differences have been observed in the gut microbiota of individuals with and without MS, [3,5-8] including pediatric MS.[5] Pediatric MS offers opportunity to study disease processes in the very early stages of MS, relatively close to the actual biological onset of disease, potentially limiting confounders. We explored the association between gut microbiota profiles in early pediatric MS and subsequent relapse risk.

Methods

Cohort selection

Children ≤18 years old with a first demyelinating event and at least 2 silent brain lesions or relapsing-remitting MS (McDonald criteria) attending a University of California, San Francisco (UCSF) pediatric MS clinic provided a baseline stool sample, as described previously.[5] At baseline, all cases were within 2 years of symptom onset with no systemic antibiotic exposure in the previous 2 months.

Capture of clinical and demographic data

Baseline characteristics captured included demographic (e.g. age, sex), and clinical (e.g. disease duration, immunomodulatory drug (IMD) exposure). After stool collection, physician confirmed relapses were determined via structured forms and chart review by abstractors unaware of the child's gut microbiota profile.

DNA extraction and 16S rRNA sequencing

DNA was extracted from stool using a cetyl trimethylammonium bromide method[9] and the V4 region of the 16S rRNA gene was amplified in triplicate,[5,10] combined, purified and pooled in equimolar concentrations prior to sequencing on the Illumina MiSeq platform. Reads were clustered at ≥97% similarity into operational taxonomic units and singly rarefied to 201,546 reads per sample. Taxonomy was assigned using the Greengenes database via QIIME (Quantitative Insights Into Microbial Ecology).[11]

Statistical analyses

The gut microbiota formed the exposure, expressed as phylum-level relative abundance and categorized according to the data distribution as either 'absent vs. present,' or 'low vs high' (≤ vs. >median) when detectable in >90% of cases. Phyla with sparse data were excluded (i.e. <20% of cases had detectable reads).

The outcome was the first on-study (post-baseline) relapse. Relapse-free cases were censored at their last clinic visit. Associations between each exposure and the outcome were explored using Kaplan-Meier curves, with the log-rank test to compare groups. After applying a conservative Bonferroni correction for multiple comparisons, those phyla remaining significant were assessed though multivariable Cox regression models, adjusting for potential confounders, including age and IMD drug exposure status (see Appendix, online).

In a sensitivity analysis, any child with an attack (either the onset attack or a relapse) within 30 days pre-stool sample was excluded and the log-rank tests were repeated.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Ver.22.0.NY:IBM Corp.2013). UCSF's Institutional Review Board approved the study.

Results

Of the originally reported 18 MS cases,[5] 17 had up to 41.6 months (mean=19.8 months) post-baseline follow-up (one left the country and was excluded). Cohort characteristics are shown in the Table, additional characteristics (e.g. diet, body mass index) are available online (Supplementary Table). During follow-up, 7 relapsed (9 relapses were recorded in total from baseline). From Kaplan-Meier curves, 25% of cases relapsed within an estimated 166 days from baseline.

Eight of 13 phyla identified had sufficient data to be examined (i.e. detected in >20% cases). A shorter time to relapse was associated with absence of *Fusobacteri*a (p=0.001, log-rank

test), a higher abundance of *Firmicutes* (p=0.003) and presence of the Archaea *Euryarchaeota* (p=0.037), see Figure. No remarkable associations were observed with the remaining phyla (Bacteroidetes, Actinobacteria, Verrucomicrobia, Proteobacteria and *Tenericutes*) and time to relapse (all p>0.05, data not shown). Only two phyla (*Firmicutes* and *Fusobacteria*) reached significance after a Bonferroni correction (p<0.00625) and Cox regression models were built for these only (see Figure). After covariate adjustments, the *Fusobacteria* phylum remained significant; its absence (vs presence) being associated with a 76% (95%CI:55%-90%) chance of an earlier relapse (HR=3.2 (95%CI:1.2-9.0), p=0.024 age and IMD exposure adjusted).

In the sensitivity analyses, exclusion of the participant with a relapse within 30 days prior to the stool sample did not change the direction of findings; the differences were more significant for *Firmicutes* (p=0.002, log-rank test) and *Fusobacteri*a (p=0.00033) but not *Euryarchaeota* (p=0.041).

Discussion

Findings suggest that gut microbiota composition may be associated with subsequent relapse risk. Absence of *Fusobacteria* was associated with over three times the hazard of an earlier relapse relative to a child with measurable levels of this phylum (see Figure). This remained significant after adjustment for potential confounders.

While data were suggestive that presence or higher abundance of *Firmicutes* and *Euryarchaeota* might also be associated with relapse, neither remained significant after either adjustment for multiple testing (*Euryarchaeota*) or potential confounders (*Firmicutes*). Nonetheless, as both these phylum have been associated with inflammatory conditions such as inflammatory bowel disease (Crohns) and obesity,[12] it may be of value to consider these in larger studies. Our findings may help in the optimal design of future studies.

The literature for *Fusobacteria*, which houses a large number a distinct species, is mixed; its presence being associated with health (in animals[13]) and disease, including colorectal cancer,[14] which interestingly, individuals with MS appear at lower risk of developing (relative to matched general population controls[15]). This suggests that additional analyses examining the specific *Fusobacteria* species associated with reduced risk of relapse is warranted.

Of the *Fusobacteria* identified, the genera were either *Fusobacterium* or *Leptotrichia* (genus was the lowest taxonomic level available). *Fusobacteria* comprise of anaerobic, gram negative bacteria, as does the major phylum *Bacteroidetes*. Members of the *Bacteroidetes* phylum, such as *Bacteroides fragilis* have been shown to ameliorate the animal model experimental autoimmune encephalomyelitis (EAE) via polysaccharide A expression.[3,4] It is possible that a similar pathway explains the observed association with the *Fusobacteria*.

Our study included children very close to disease onset, i.e. the onset attack. It remains possible that there were residual effects on the gut microbiota from previous (pre-baseline) attacks (including the onset attack). Longitudinal stool sampling, combined with clinical

data, are required to understand the impact of sequential relapses. It is possible that those with frequent relapses are caught in a self-perpetuating cycle of inflammation in which the gut microbiota represents a propagating factor and reservoir of pro-inflammatory signaling. Nonetheless, removing those with a relapse within 30 days pre-stool did not change the direction of findings. It is not possible to determine whether the relative absence of *Fusobacteria* resulted from outgrowth of another competing microbe or direct demise of multiple members of this phylum. A better understanding of the gut microbiota's role in modifying MS relapse risk may identify novel drug targets and improved outcomes in MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Berer K, Mues M, Koutrolos M, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature. 2011; 479:538–41. [PubMed: 22031325]
2. Lee YK, Menezes JS, Umesaki Y, Sarkis K, Mazmaniana SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA. 2011; 108(Suppl 1):4615–4622. [PubMed: 20660719]
3. Mielcarz DW, Kasper LH. The Gut Microbiome in Multiple Sclerosis. Curr Treat Options Neurol. Apr.2015 17(4):344. doi: 10.1007/s11940-015-0344-7. [PubMed: 25843302]
4. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, Burroughs AR, Begum-Haque S, Dasgupta S, et al. Central nervous system demyelinating disease protection by the human commensal Bacteroides fragilis depends on polysaccharide A expression. J Immunol. 2010; 185:4101–4108. [PubMed: 20817872]
5. Tremlett H, Fadrosh DW, Faruqi AA, et al. Gut microbiota in early pediatric multiple sclerosis: a case-control study. 2016 Under review.
6. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, et al. Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. PLoS One. 2015; 10(9):e0137429. [PubMed: 26367776]
7. Cantarel BL, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J, et al. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. J Invest Med. 2015; 63(5):729–34.
8. Rumah KR, Linden J, Fischetti VA, Vartanian T. Isolation of Clostridium perfringens type B in an individual at first clinical presentation of multiple sclerosis provides clues for environmental triggers of the disease. PLoS One. 2013; 8(10):e76359. [PubMed: 24146858]
9. DeAngelis KM, et al. Selective progressive response of soil microbial community to wild oat roots. ISME J. 2009; 3:168–78. [PubMed: 19005498]
10. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012; 6:1621–4. [PubMed: 22402401]
11. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 2010; 7:335–6. [PubMed: 20383131]

12. López-Cepero AA, Palacios C. Association of the Intestinal Microbiota and Obesity. P R Health Sci J. 2015; 34:60–4. [PubMed: 26061054]

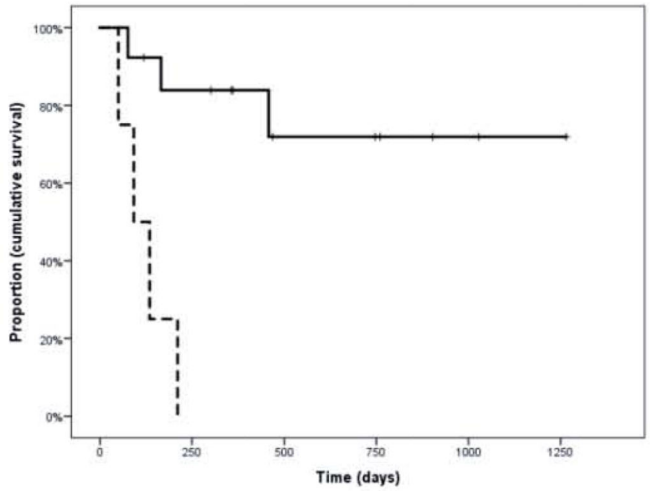
13. Suchodolski JS, Dowd SE, Wilke V, Steiner JM, Jergens AE. 16S rRNA gene pyrosequencing reveals bacterial dysbiosis in the duodenum of dogs with idiopathic inflammatory bowel disease. PLoS ONE. 2012; 7:e39333. [PubMed: 22720094]

14. Mima K, Nishihara R, Qian ZR, et al. Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. Gut. 2015 Epub ahead of print.

15. Kingwell E, Bajdik C, Phillips N, et al. Cancer risk in multiple sclerosis: findings from British Columbia, Canada. Brain. 2012; 135:2973–2979. [PubMed: 22730559]

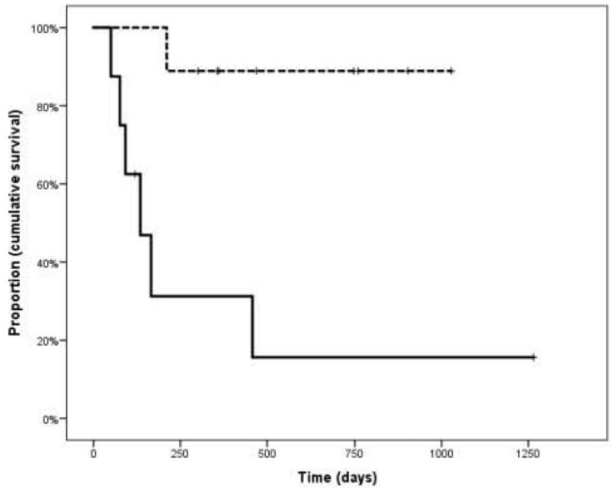
Highlights

- Gut microbiota was associated with subsequent relapse risk in pediatric multiple sclerosis
- *Fusobacteria* depletion increased relapse risk (hazard ratio=3.2;95%CI:1.2-9.0)
- Findings could offer new targets to alter the MS disease course



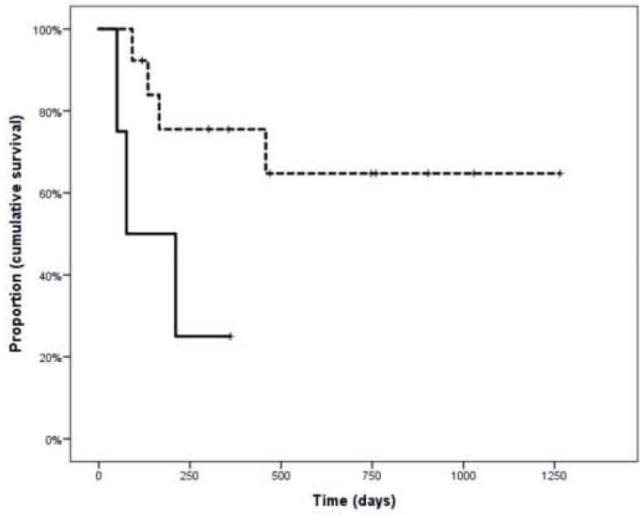
Risk of relapse (Cox regression)

Hazard ratios (95%CIs)	***Fusobacteria*** [Reference=present]
Unadjusted	**3.4 (1.4-8.2), p=0.005**
Age-adjusted	**3.3 (1.2-8.8), p=0.019**
Age & IMD adjusted	**3.2 (1.2-9.0), p=0.024**



Risk of relapse (Cox regression)

Hazard ratios (95%CIs)	***Firmicutes*** [Reference='low']
Unadjusted	**13.4 (1.6-114.7), p=0.018**
Age-adjusted	5.6 (0.4-69.5), p=0.184
Age & IMD adjusted	6.2 (0.4-87.2), p=0.177



Failed to reach significance based on Bonferroni corrected p-value ($p>0.00625$) such that a multivariable Cox model was not fitted

Figure 1. Association between gut microbiota (phylum-level) and relapse for: *Fusobacteria* [panel A, top], *Firmicutes* [panel B: middle] and *Euryarchaeota* [panel C, bottom]

Panel A: *Fusobacteria:* Kaplan-Meier curves (left), absent (dashed line) vs. present (solid line). p=0.001, log-rank test.

Panel B: *Firmicutes:* Kaplan-Meier curves (left), lower relative abundance (dashed line) vs. higher (solid line), p=0.003. log-rank test.

Panel C *Euryarchaeota:* Kaplan-Meier curves (left), absent (dashed) vs present (solid linegreen), p=0.037, log-rank test.

Key: IMD=immunomodulatory drug.

Hazard ratios indicate risk of relapse from baseline and are derived from Cox regression hazards models. Age at baseline (stool collection) and IMD exposure at baseline (exposed vs. naïve) were used to adjust models as shown**. Bold indicates p<0.05.**

Binomial categories for each phylum were created based on the data distribution as either absent versus present or high versus low (≤ vs. > median relative abundance). Of the Fusobacterium phyla identified, the genera were either *Fusobacterium* or *Leptotrichia* (genus was the lowest taxonomic level available).

Table 1

Baseline characteristics of the pediatric multiple sclerosis (MS) cases[φ]

Characteristic, n (%) unless stated otherwise	MS cases, n=17
Sex: Girl	10 (59%)
Boy	7 (41%)
Age, years: mean (SD; range)	12.5 years (SD=4.57; 4-17)
Age: ≤12 years old	5 (29%)
>12 years old	12 (61%)
Race: White	8 (47%)
Non-white	9 (53%)
Ethnicity: Hispanic	8 (47%)
Non- Hispanic	9 (53%)
Co-morbid condition [a]: present	7 (41%)
Absent	10 (59%)
MS-Specific clinical characteristics	
Age at MS symptom onset, years: mean (SD; range)	12.1 years (SD=4.8; 4-17)
Disease duration [b], months: mean (SD; range)	10.3 months (SD=6.6; 2.3-23.1)
Time since last relapse or onset attack (onset attack considered): days: mean (SD; range)	183 days (SD=140; 4 to 489 days)
Disability level - EDSS at enrolment, median (range)	2.0 (0-4.0)
0-<2.0	7
2.0-<3.0	7
3.0+	3
Immunomodulatory drug exposure status [c]: IMD naïve	8 (47%)
IMD exposed	9 (53%)
Corticosteroids – systemic [d]: No	11 (65%)
Yes	6 (35%)
Available prospective follow-up[ǂ], months: mean (SD; range)	19.8 months (SD=12.0; 1.8-41.6)

Key: SD=standard deviation; EDSS=Expanded Disability Status Scale score; IMD=immunomodulatory drug

[φ]data shown are in relation to baseline (i.e. date of stool sample collection) unless otherwise stated. EDSS was assessed at the clinic visit nearest to the stool sample, i.e. at enrollment into the study

[ǂ]all prospective follow-up was expressed regardless if (or when) a relapse occurred, with the study end being the last clinic visit or contact for each child

[a]the comorbid conditions for the 7 children were: headache, atopic dermatitis/eczema, long-term constipation, history of shingles, seizures, reactive airways disease and headache, scoliosis

[b]disease duration: time from symptom onset to baseline (stool collection)

[c] 'IMD naïve' indicates never exposed pre-baseline. 'IMD exposed' indicates ever exposed pre-baseline. At baseline, all IMD exposed cases were still on an MS drug as follows: beta-interferon (n=3); glatiramer acetate (n=5); natalizumab (n=1). No child had switched or stopped an IMD (although one child had previously been exposed to plasma exchange before taking glatiramer acetate).

[d] within the previous 2 months