a place of mind

Gut microbiome in early pediatric multiple sclerosis: a case-control study

H Tremlett [1], D Fadrosh [2], Ali A Faruqi[2], F Zhu[1], J Hart[2], J Graves[2], S Lulu[2], G Aaen[3], A Belman[4], L Benson[5], C Casper[6], T Chitnis[7], M Gorman[5], L Krupp[4], TE Lotze[8], J Ness[9], S Roalstad[6], M Rodgriguez[10], J Rose[6], J-M Tillema[10], B Weinstock-Guttman[11], S Lynch[2], E Waubant[2], and the US Network of Pediatric MS Centers www.usnpmsc.org Email: helen.tremlett@ubc.ca; Emmanuelle.Waubant@ucsf.edu

[1]Uni. British Columbia, Vancouver, Canada, [2]Uni. California, San Francisco, USA, [3] Loma Linda Uni., CA, USA, [4] Stony Brook Uni., NY, USA, [5] Boston Children's Hospital, Harvard Medical School, Boston, MA, USA, [6] Uni.Utah, Salt Lake City, UT, USA, [7] Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [8] Baylor College of Medicine, Houston, TX, USA, [9] Uni. Alabama, Birmingham, AL, USA, [10] Mayo Clinic, Rochester, MN, USA, [11] State Uni. New York at Buffalo, USA

Introduction

- Alterations in the gut microbiome have been associated with neurological and autoimmune diseases, including Multiple sclerosis (MS) [1-4]
- The gut microbiomes' diverse roles range from vitamin synthesis to immune system modulation [5]
- Pediatric MS, offers a unique opportunity to explore the microbiome close to the actual onset of disease, after relatively few years of life and exposures have accrued

Objectives

To examine the gut microbiome in patients with early onset pediatric MS and control subjects of similar age and sex. To assess whether demographic or disease features were associated with the gut microbiome.

Patient Data and Methods

Eligibility:
Children ≤18 years attending a University of California, San Francisco, USA paediatric clinic
Cases: MS (McDonald criteria 2010); within 2 years of symptom onset
Controls: without autoimmune disease (asthma or eczema allowed); neither parent had MS or related disorders

Table 1. Select characteristics of children with early onset MS and controls

Characteristic	MS case, n=18	Control, n=17 [a]
Sex, n (%): Girl	10 (56%)	9 (53%)
Age at stool sample , mean (SD; range)	12.5 years (SD=4.44; 4-17)	13.5 years (SD=3.08 ; 9-18)
Race: White	9 (50%)	13 (77%)
Ethnicity: Hispanic	8 (44%)	6 (35%)
Overweight or obese (≥85th percentile[b])	6 (33%)	5 (29%)
High-fat, low fibre diet [c]	5 (28%)	5 (29%)
Mode of delivery: vaginal	16 (89%)	15 (88%)

Key: [a] 16/17 controls contributed to the sequencing and 14/17 controls to the microarray
[b] from age-sex BMI growth charts. BMI and mode of delivery missing for one control child
[c] Diet metrics derived from the Block Kids Food Screener (NutritionQuest) [6].

All cases had early onset RRMS
- age at symptom onset: 12.1 years (mean, range: 4-17)
- disease duration at stool sample: 10.6 months (mean; range: 2-23)
- 9 (50%) exposed to an immuno-modulatory drug [IMD]: 5 glatiramer acetate, 3 beta-interferon, 1 natalizumab
- 6 exposed to a corticosteroid (2 months pre-stool sample)

No case or control exposed to an antibiotic with 2 months pre-stool

Sample collection, processing and analyses:
Stools were shipped on ice and stored at -80C until DNA extraction. Full-length 16S rRNA gene (27F.1/1492.jgi) was amplified and assessed using Illumina Miseq with primers for the V4 hyper variable region (515F-806R). The G3 PhyloChip microarray (Second Genome, Inc., CA) formed a secondary, validation platform. Taxa (OTU) were defined by 16S rRNA sequences with ≥97% similarity. Associations between the patient characteristics and community composition were explored using permutational multivariate analysis of variance. Rate ratios were calculated via negative binomial regression. Microbiome functionality was inferred via PICRUSt [7]

Literature: 1. Wang Brain Behav Immun. 2013. 2. Berer FEBS Lett 2014. 3. Berer Nature 2011. 4. Rumah PLoS One 2013. 5. Kau. Nature 2012. 6. Block J Am Diet Assoc 2002. 7. Langille Nature Biotech 2013

Special thanks to all participating children and their parents/guardians. Tom Duggan for Figure 1

Study specific funding: **National MS Society** RG4861A3/1 (PI Waubant), **National Institutes of Health** NS071463 (PI Waubant). **The Race to Erase MS** (PI Waubant). No authors had a COI directly related to this study. For all disclosures, please see abstract book.

Results – sequencing platform

Overall gut microbiome composition was similar for cases vs. controls; but differed for other characteristics

- **Cases and controls were similar for alpha and beta diversity metrics**: richness, evenness, Faith's phylogenetic diversity and Canberra distance matrix (p>0.1; Mann-Whitney)
- Of the other characteristics explored [Table 1], gut microbiome composition was most significantly related to:
- **Ethnicity:** hispanics exhibited lower alpha diversity than non-Hispanics, reaching significance for evenness only (Mann-Whitney, p=0.040)
- **IMD exposure:** explained 6.8% of the variation (Canberra distance; IMD exposed vs unexposed cases vs controls, p=0.012) or 7.1% within the MS cases only (IMD exposed vs unexposed cases, p=0.016).

Specific taxa were significantly enriched and depleted in MS versus control subjects

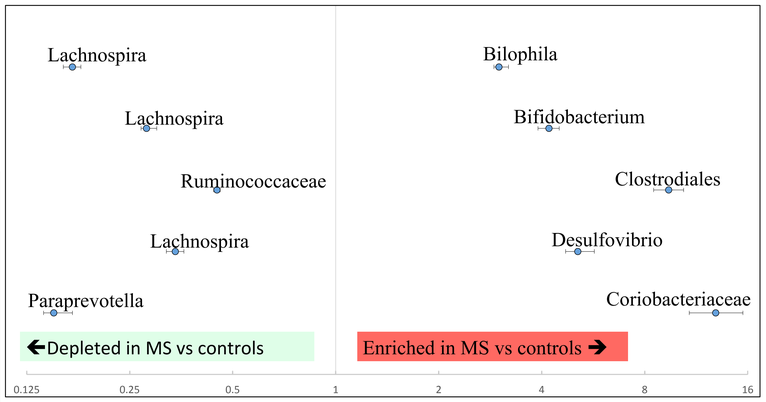


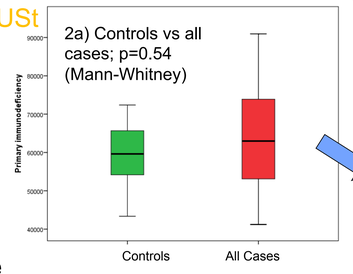
Figure 1. Top 10 most significantly enriched and depleted taxa MS (vs controls): rate ratios (95%CIs)

- Of 25,134 taxa identified, 160 were significantly enriched in MS cases; 163 were depleted
- Figure 1 shows the top 5 most significant for each (all p and q <0.000001)

Example: Children with MS had 3.0 times the abundance of Bilophila (95%CI: 2.9-3.2) than control children

Predicted community function using PICRUSt

- Multiple pathways (KEGG orthologs) were enriched in cases vs controls, e.g. folate biosynthesis, glutathione metabolism and the renin-angiotensin system (Mann-Whitney, all p<0.05)
- Findings were affected by IMD exposure, see Figure 2a,b for an example



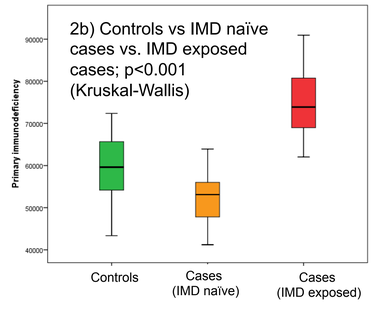


Figure 2a, b. Example of a pathway where findings were influenced by IMD exposure: primary immunodeficiency

- Microarray findings broadly concurred with sequencing (alpha and beta diversity; data not shown)

Conclusions

- Although overall gut community composition did not differ significantly between MS case and controls, specific taxa were significantly altered in relative abundance in this very early onset pediatric MS cohort
- Suggests that MS onset may be associated with changes in a few taxa rather than overall community composition
- IMD exposure explained the greatest compositional variance in the microbiome in this cohort

Future directions: Findings require further interrogation, analysis and confirmation, but will guide the design of future structural and functional studies