What is in your gut? The CHARGE Microbiome

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INTRODUCTION

- Difficulties with feeding and digestion are common in individuals with CHARGE syndrome. The impact of gastrointestinal (GI) distress is considered similar in severity to patients with inflammable bowel disease (IBD)[1].

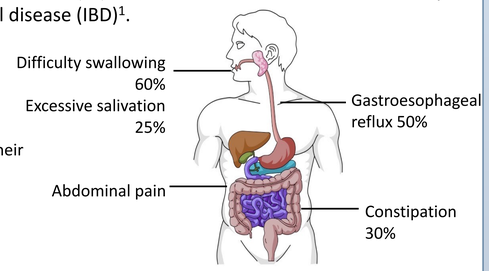


Figure 1: Commonly reported GI issues in Individuals with CHARGE syndrome and their prevalence where applicable[2,3]

- The chromodomain helicase DNA binding protein 7 (Chd7) mutation found in CHARGE syndrome play a role in neural crest development. Research shows zebrafish with chd7 mutations also have fewer enteric and vagus nerves innervating the gut[4]. This leads to dysregulated gut contractions and delayed emptying in the zebrafish model[5].
- In addition to central control and nerves, gut health is regulated by the colonies of bacteria that inhabit the GI tract. Trillions of bacteria live in the intestines, known as the gut microbiome. There they aid in metabolism, regulate the immune system, and influence cognitive behaviour through the gut-brain axis.
- Imbalances in the type and number of bacteria, called gut dysbiosis, has been implicated in a number of health conditions such as IBD, IBS, autism, obesity, and cancer[6,7].

OBJECTIVES

1) Does the gut microbiome in individuals with CHARGE syndrome differ compared to their unaffected sibling?

2) Are there any characteristics that are common in the gut microbiome of people with CHARGE syndrome?

METHODS

- This pilot case-control study included pediatric patients across Canada with a confirmed genetic diagnosis of CHARGE syndrome, and when possible their unaffected sibling.
- Participants were asked to complete the following:

Stool Sample
- DNA was isolated and underwent high throughput sequencing to identify all 16S ribosomal RNA. This is present in all bacteria and used to differential bacteria based on unique sequences named operational taxonomic units (OTUs). This data is used to determine alpha diversity and identifies the bacterial taxonomy and relative abundance

GI Questionnaires
- **PEDSQL Gastrointestinal Symptoms Scale:** scores 10 domains of GI symptoms from 0 (never) to 4 (almost always) for a total of 232 points
- **Pediatric Assessment Scale for Severe Feeding Problems (PASSFP)**:scored from 0-64, measures feeding development, lower score = more severe feeding issues

Block Food Frequency Screener
- Records types and amount of food consumed in the week prior to stool collection. Macronutrient data is analyze by NutriQuest

RESULTS

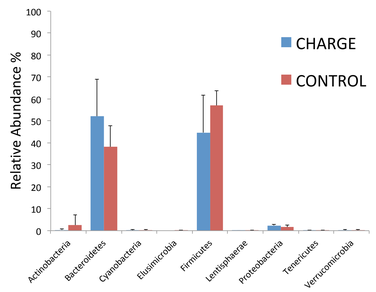
- In total, 10 participants completed the study between the age of 2-18. In the CHARGE cohort, 4 were females and 2 were males. The sibling control group had 3 males and 1 female. There were 4 sibling pairs total.
- Preliminary data analysis compared relative abundance (%) of bacteria at the level of phylum in the pooled CHARGE group versus the pooled control group. Alpha diversity (operational taxonomic units) was also observed.

Figure 2. **Average relative abundance (%) of bacteria phyla found through high throughput sequencing of stool DNA in individuals with CHARGE (n=6) and sibling controls (n=4)** (a). Table contains averages, SD, and p-values. Bacterial abundance was compared in the two groups using two-tailed t-test. Means are not significantly different (p-value < 0.05). (b) Graph displays the trend towards ↑Bacteroidetes ↑Proteobacteria and ↓Firmicute in the CHARGE cohort (blue) compared to siblings (red).

(a)

Phyla	Mean Relative Abundance (%)		Standard Deviation		p-value
	CHARGE	control	CHARGE	control	
Actinobacteria	**0.349**	**2.518**	**0.455**	**4.637**	**0.274**
Bacteroidetes	**52.058**	**38.194**	**16.896**	**9.579**	**0.090**
Cyanobacteria	0.222	0.265	0.329	0.307	0.420
Elusimicrobia	0.000	0.047	0.000	0.094	0.121
Firmicutes	**44.688**	**57.022**	**16.959**	**6.668**	**0.105**
Lentisphaerae	0.022	0.085	0.063	0.169	0.225
Proteobacteria	**2.401**	**1.623**	**0.533**	**0.917**	**0.062**
Tenericutes	0.066	0.055	0.162	0.106	0.454
Verrucomicrobia	0.190	0.191	0.262	0.354	0.498

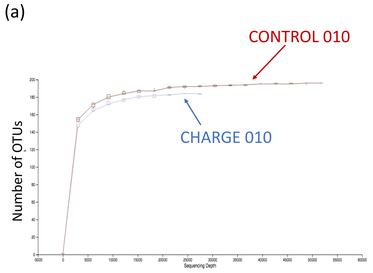
(b)



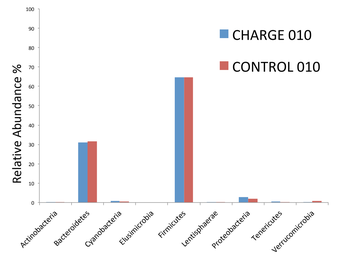
- Individual case studies for bacterial abundances were performed for each sibling pair taking difference in alpha diversity and PASSFP/PEDSQL scores into consideration.

Case 1: **Comparison of one sibling pair with similar GI symptom scores on PASSFP and PEDSQL.** (a) **Alpha diversity is similar** in individual with CHARGE and their sibling, as determined by number of operational taxonomic units identified during sequencing. (b) **Gut bacteria phyla abundances are also similar** in this sibling pair.

(a)

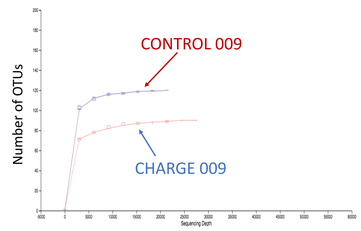


(b)



Case 2: Comparison of one sibling pair with CHARGE participant scoring high on GI symptoms and the sibling control scoring low. (a) **Alpha diversity is lower** in individual with CHARGE relative to their sibling, as determined by number of operational taxonomic units identified during sequencing. (b) **Gut bacteria phyla abundances** also show overall trend for ↑Bacteroidetes ↑Proteobacteria and ↓Firmicute.

(a)



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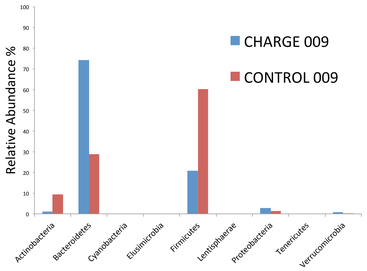

CONCLUSIONS and FUTURE WORK

- Our preliminary data shows a trend towards ↑Bacteroidetes ↑Proteobacteria and ↓Firmicute abundances in the CHARGE gut microbiome
  - Same trend was observed in similar experiment done in Austism[7]
  - ↑Proteobacteria and ↓Firmicute: often seen in IBD[6]
  - ↑Bacteroidetes: reported in IBS and chronic constipation[8]
  - Probiotic therapy has been shown to reduce anxiety-related behaviours in animals with vagus nerve dysfunction[9]
- This difference is more marked when the individual with CHARGE has more severe feeding and GI issues
- Alpha diversity, or measure of microbiome richness, tends to be similar in sibling pairs with similar feeding and GI scores, but tends to be lower if the affected individual has worse outcomes on PASSFP and PEDSQL
  - Lower diversity is associated with decreased tolerance for pathogens and perturbations in the gut[6]
- CHARGE syndrome displays a large phenotypic range and the gut microbiome is strongly influence by genetics. This enhances the importance of analyzing the data using different parameters.
- Future work will consist of further comparisons such as:
  - at level of species (Bifidobacterium and Lactobacillus)
  - feeding (oral versus G/J tube)
  - diet (high versus low fibre)
  - GI symptom type and severity
- We will increase sample size through international recruitment to increase the power and to allow for inferred metabolic activity studies based on bacteria profiles.

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