Analyzing β-glucuronidase expression in gut microbial populations of MS patients and healthy controls

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Abstract

Multiple sclerosis (MS) is a chronic and progressive autoimmune disorder of the central nervous system (CNS). Studies have observed specific differences in the microbiota of MS patients and healthy controls. A clear link between estrogen variants and MS by observing a gender bias and demonstrating that estrogens have several immunoprotective and anti-inflammatory properties. β-glucuronidase is an exogenous enzyme that degrades estrogens into a non-invasive form and such, may contribute to protection against MS. There has yet not been a study that characterizes the interplay between estrogen variants, β-glucuronidase, and the gut microbiome but doing so may help elucidate hormonal and immunological mechanisms implicated in multiple sclerosis pathology. We hypothesize that the relative gut microbial β-glucuronidase gene counts for healthy controls will be greater compared to that for MS patients. To test this hypothesis we assessed the estrobolome to analyze the levels of β-glucuronidase gene expression in control and MS patients. Using data obtained from previous clinical studies, we characterized microbiota populations through the QIIME2 pipeline and performed a predictive metagenomic analysis using PICRUSt2 plugin to generate β-glucuronidase gene counts and allow for pairwise comparison between MS patients and healthy controls. We were unable to confirm our hypothesis that gut microbial β-glucuronidase gene counts for healthy controls are greater than that for MS patients. To quantitatively identify several microbial populations that could be correlated to MS pathogenesis which may prove worthwhile to further investigate.

Introduction

Multiple sclerosis (MS) is a chronic and progressive autoimmune disorder of the central nervous system (CNS). Studies have observed that there are specific microbial taxonomic differences in the gut microbiota of MS patients when compared to healthy controls. The factor driving the gender bias characteristic of RRMS and its onset overall may be attributed to the influence of gut microbes on the endocrine-based autoimmune. There is clinical evidence that identifies a clear link between estrogen variants and microbiome differences in biological and clinical outcomes (E2 and E3) are endogenous estrogens produced in humans. E2 inhibits pro-inflammatory cytokines and E3 has been shown to reduce EAE severity in murine models. A randomized, placebo-controlled, double-blind trial conducted with female RRMS patients and E3 found that continued relapse rate was lower in the E3-treated RRMS groups. As such, lower concentrations of active circulating estrogens may have a pro-inflammatory background for MS pathogenesis. Here, we focus on a subset of the microbiome called the estrobolome which concerns bacteria that are capable of modulating or metabolizing the body’s circulating estrogens. Bacteria in the estrobolome regulate circulating estrogen levels by secreting β-glucuronidase, an enzyme that degrades estrogens into their active form. Subsequent dysbiosis of these gut microbes may be correlated with MS pathogenesis.

Hypothesis - The relative gut microbial β-glucuronidase gene counts and by extension, gene expression, for healthy controls will be greater compared to that for MS patients.

Materials & Methods

Specific Aims - Aim 1: Identify what microbial populations are associated with RRMS and those that express the β-glucuronidase gene. Aim 2: Identify what microbial populations in the estrobolome express the β-glucuronidase gene by assessing DNA associated with RRMS. Aim 2: Identify what microbial populations in the estrobolome express the β-glucuronidase gene by assessing DNA associated with RRMS.

We used the QIIME2 pipeline to identify microbial populations in the datasets retrieved from the two clinical studies, which contained raw 16s rRNA sequences. 1, 2 We imported the sample data and screened them using DADA2 for demultiplexing and de novo sequencing before using the pipeline to align the resulting sequences with the GreenGenes database. This enabled us to quantify core metrics like alpha and beta diversity while also developing a phylogenetic tree to identify observed OTUs as well as their differential abundance. For alpha diversity, we used the Faith’s phylogenetic distance between sets of taxa based on mutual lineages while taking into account degrees of similarity among 16s rRNA sequences.

With the alignment data, we then used the PICRUSt2 plugin in QIIME2 to perform a predictive analysis on the raw 16s rRNA sequences. Both of these analyses identify core β-glucuronidase gene counts specific to each microbial community present for pairwise comparison between MS patients and healthy controls.

Results

Discussion

In this study, we explored and characterized the estrobolome in RRMS and control patients and the impact of the expression of the β-glucuronidase gene on MS pathogenesis. We identified microbial populations associated with RRMS and those that express the β-glucuronidase gene by performing a pairwise analysis of β-glucuronidase gene count between healthy controls and MS patients. This was done using datasets from two previous clinical studies and by taking advantage of bioinformatics platforms like QIIME2 to assess differences in microbial communities and structure and other state-of-the-art pipelines like PICRUSt2 for predictive metagenomic analysis of the β-glucuronidase gene.

We first assessed the alpha diversity of our samples obtained from the two clinical studies, one dataset generated from 16s sequencing and the other from whole genome sequencing, using the Faith, Shannon, and Observed Features metrics up to a sequencing depth of 50,000 and were able to confirm observations made by other studies that similar microbial populations are present in the gut microbiomes of MS patients and healthy controls (Figure 1a). And in assessing beta diversity, we observed a significant phylogenetic difference in microbial community structure between the microbiota of MS patients and that of healthy controls than the microbiota of healthy controls with that of other healthy controls (Figure 1b). Further, since there were differences in microbial community structure, we then assessed the microbial composition of each subject to identify specific taxonomic groups present in each subject and their differential abundance (Figure 2). With this and a further analysis of the differential abundance of these OTUs, we were able to qualitatively and quantitatively identify several microbial populations that were more abundant in either MS patients or healthy controls (Figure 3). Notable OTUs were observed in the genera in the families in the genus Butyrivibrio and notable OTUs abundant in MS patients include Akkermansia muciniphila and Ruminococcus flavefaciens.

A caveat of our analyses is that we were limited by the computing power of our personal computers and bottlenecked by downloading large files for other analyses. We were thus unable to perform whole genome analysis of the alignment data using MetaPhlAn and HUMAnN on the confidential microbiome. A potential future direction for this study would be to secure increased computing power in order to consider more extensive predictive analyses, analyze the whole genome data sets, and further analyze the impact of selected OTUs. It may prove worthwhile to study these microbial populations in greater detail to better understand their physiological significance in the human body, particularly in the estrobolome, as they may be correlated with MS pathogenesis or confer protection against MS.

Selected References


See full paper for a comprehensive list of references.