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Short communication

Are the decrease in circulating anti-α1,3-Gal IgG and the lower content of galactosyl transferase A1 in the microbiota of patients with multiple sclerosis a novel environmental risk factor for the disease?

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ABSTRACT

The etiology of multiple sclerosis (MS), particularly the environmental component of the disease, remains speculative. Recent reports have suggested that alterations in the gut microbiota of MS patients could contribute to the etiology or pathophysiology of the disease. In this Viewpoint, using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to infer the functional content of the gut microbiota, we show that the gut microbiota of MS patients is characterized by a significant decrease in the relative abundance of the enzyme EC 2.4.1.87, which corresponds to the GGTA1 gene (which codes for the α1,3-Gal epitope and is lacking in humans), against which MS patients also have low levels of IgG antibodies. The decrease in circulating anti-α1,3-Gal IgG and lower content of galactosyl transferase A1 in the microbiota of patients with multiple sclerosis could be a novel environmental risk factor for the disease.

1. Introduction

The amount and taxonomic composition of the intestinal microbiota affect the host immune response in experimental models (Berer and Krishnamoorthy, 2014). Gut microbiota patterns from patients suffering from multiple sclerosis (MS) differ from those of healthy individuals. MS patients have reduced relative abundances of *Bacteroidaceae*, *Bacteroides*, *Parabacteroides, Faecalibacterium*, *Prevotella*, *Anaerostipes*, *Collinsella* and *Slackia* (Miyake et al., 2015; Cantarel et al., 2015; Chen et al., 2016a; Jangi et al., 2016), which are believed to affect host immunity (Kamada et al., 2013).

Recently, we reported that MS patients exhibit a decrease in anti-α1,3-Gal IgG blood levels compared with those of age/gender-matched healthy individuals (Le Berre et al., 2017). The α1,3-Gal epitope is lacking in human glycans following the loss-mutation of the glycosyltransferase A1 (GGTA1) gene that controls its synthesis (Padler-Karavani et al., 2008). Anti-α1,3-Gal antibodies, which appear during the first year of life, supposedly due to the immunization against gut microbiota (Cooper et al., 1994), represent a substantial fraction of the total IgG and IgM pool in humans (up to 1 percent of B cells display a B cell receptor committed to α1,3-Gal) (Galili, 2013). The levels of anti-α1,3-Gal antibodies may affect the immune response against GGTA1-positive infectious agents by several mechanisms (Galili, 2016; Soares, 2015) and may also be involved in autoimmune processes (Galili, 2013).

In this Viewpoint, we suggest that the abnormal level of anti-α1,3-Gal antibodies could be related to abnormal amounts of GGTA1 gene-positive microorganisms in the MS patient microbiota, providing a new link to novel alterations in environmental factors in the disease (working hypothesis in Fig. 1A).

2. Methods

To test our hypothesis, we obtained the publicly accessible raw 16S ribosomal (r) RNA data from 2 recently published studies with publicly available data sets. 1) Chen et al. investigated the gut microbiota in relapsing remitting MS (RRMS) (n = 31) patients and in age- and gender-matched healthy controls (n = 36). Taxonomic profiles were generated by sequencing the V3–V5 region of the 16S rRNA on the MiSeq platform (Illumina) (Chen et al., 2016a). 2) Jangi et al. investigated the gut microbiota in MS (n = 60) patients and healthy controls (n = 43). The V4 region of the 16S rRNA was sequenced on the MiSeq platform (Illumina) (Jangi et al., 2016).

The 16 S rRNA sequencing data from the Illumina runs were

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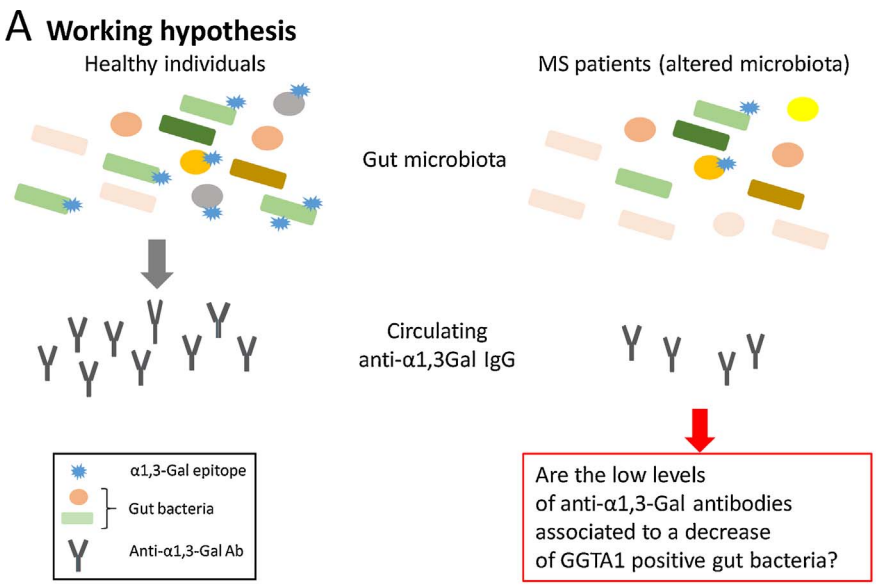
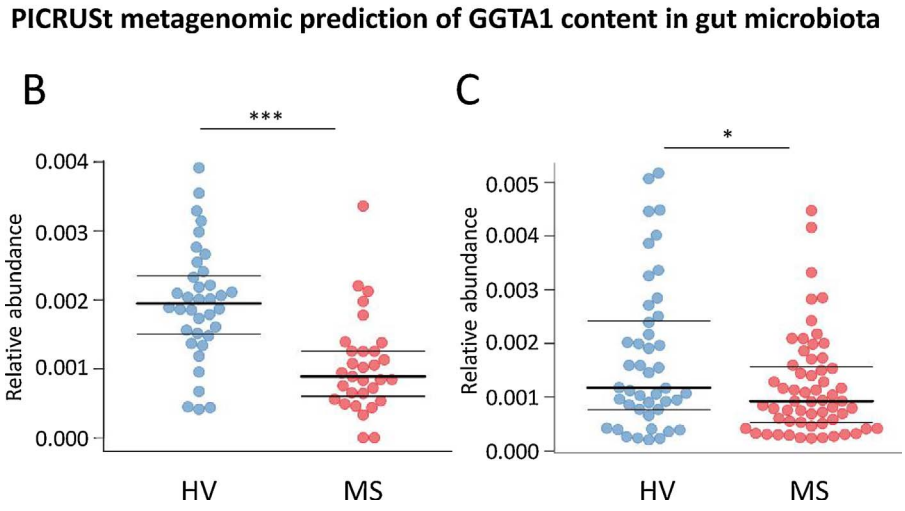


Fig. 1. (A) This panel summarizes the working hypothesis. MS patients exhibit an altered gut microbiota (Chen et al., 2016a; Jangi et al., 2016) and a decrease in circulating anti-α1,3 Gal IgG (Le Berre et al., 2017). In healthy individuals, these antibodies are produced in response to gut bacteria expressing the enzyme α1,3-galactosyltransferase (Galili, 2013). Are the low levels of anti-α1,3 Gal IgG in MS patients associated with a decrease in GGTA1 (gene encoding α1,3-galactosyltransferase)-positive bacteria in the gut? (B) and (C) Relative abundance of EC 2.4.1.87 enzyme (the GGTA1 gene controlling α1,3-galactosylation) based on PICRUSt metagenome prediction in MS patients and healthy volunteers (HV) matched for age and sex. (B) From Chen et al. (Chen et al., 2016a). Grubbs analysis did not identify any outliers. The difference between the 2 groups of subjects was highly significant (t-test, *p*-value = 7.2e-06). (C) From Jangi et al. (Jangi et al., 2016). The Grubbs test identified 2 outliers in the HV cohort, and these outliers were excluded from the analysis. The difference between the two groups of subjects was statistically significant (t-test, *p*-value = 0.0263).



trimmed and filtered to remove chimeras using Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1 (Caporaso et al., 2010) with quality filtering steps for sequence length, end-trimming, and minimum quality score. Preprocessed sequences were clustered at the 97% nucleotide sequence similarity level, using closed-reference Operational Taxonomic Unit (OTU) picking against the Greengenes 13_8 reference database (DeSantis et al., 2006).

We then used PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to predict the functional composition of a metagenome using the 16S rRNA gene and a database of reference genomes. PICRUSt uses ancestral state reconstruction to infer the content of the organism's last phylogenetic common ancestor based on one or more sequenced genomes, along with a weighting method (maximum likelihood or Bayesian posterior probability), to predict the gene content for all organisms represented in the Greengenes phylogenetic tree of 16S sequences. This metagenomic approach recaptures key findings from the Human Microbiome Project and accurately predicts the actual abundance of gene families in host-associated and environmental communities with a high degree of confidence (Langille et al., 2013). The final output from PICRUSt was an annotated table of predicted gene family counts for each sample with a detailed KEGG Orthology (KO) description and with enzyme entry annotation. Based on the reference database ExPASy, GGTA1 corresponds to the enzyme entry 'EC 2.4.1.87' (http://enzyme.expasy.org/EC/2.4.1.87). We also searched for outliers in each dataset using the Grubbs test from the *outliers* package in the R statistical software (R Development Core Team, Vienna, Austria). We then compared the relative abundance of the EC 2.4.1.87 enzyme in MS patients and controls using a *t*-test in the R software. Interestingly, PICRUSt also estimates the contribution of each OTU to a given gene function. We used *metagenome_contributions.py* from PICRUSt, which partitions the predicted contribution to the metagenomes from each organism in the OTU table, limited to the EC 2.4.1.87 enzyme.

3. Results

We first analyzed the 16 S rRNA raw data from Chen et al., who investigated intestinal microbiota alterations in MS patients compared to age/gender-matched healthy controls (Chen et al., 2016a). Using PICRUSt, we produced a metagenome prediction table with enzyme entry annotations based on the closed-reference OTU table. There were no outliers in the two groups of subjects in terms of the EC 2.4.1.87 level. The relative abundance of EC 2.4.1.87 was very significantly decreased in MS patients compared to the controls (t-test, *p*-