The Gut Virome of Multiple Sclerosis Changes in Distinct Phage Groups Identified by Exploratory Factor Analysis PDF poster

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Abstract

Background: Bacteria and viruses are the major microbes in gut microbiota; dysbiosis, an imbalance in the bacterial populations, has been demonstrated in various diseases, including multiple sclerosis (MS), by standard methods, such as relative abundance of bacteria and component/coordinate analysis (PCA/PCoA). These methodologies failed to character.

Objective: We aimed to establish a bioinformatics method to analyse the virome and characterize the gut virome of MS, using exploratory factor analysis (EFA). EFA has been widely used in the psychological field; EFA can identify the nature and number of common factors among the variables.

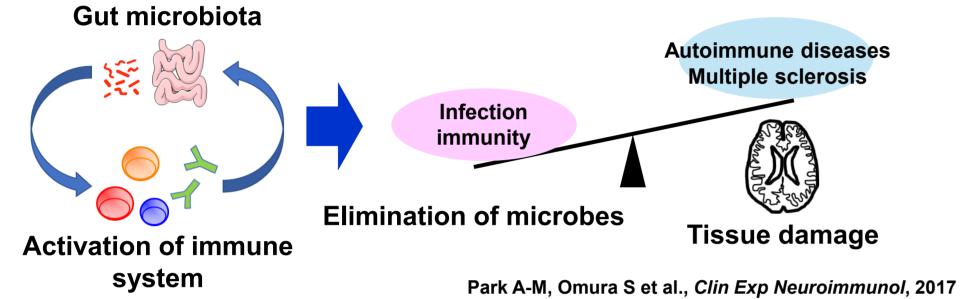
Methods: We used shotgun metagenome data of fecal DNA samples from relapsing-remitting MS (n=45) and controls (n=51) from a database (Accession no. PRJNA1084182, Ghimire, 2025), where we extracted virome data. Using R, we found that PCA did not distinguish MS and control virome data. Thus, we analysed the data by EFA using the same method as we published in Guillain-Barré syndrome research; previously, we classified 55 anti-glycolipid antibodies by EFA and identified five latent factors associated with pathogenesis (Omura,

Results: Using both MS and control virome data, we extracted 1,525 species of DNA viruses/bacteriophages (phages). EFA extracted six factors from the virome data. Factor 1 contained 269 phages infecting Gram-positive bacteria, including the genus Propionibacterium. Factor 2 contained 117 phages infecting Gram-negative bacteria, including the genera Escherichia and Pseudomonas. Factor 3 contained 332 phages, whose host bacteria included the genera Enterococcus and Salmonella. All viruses in Factor 4 were 113 Streptococcus phages belonging to the family Aliceevansviridae. Factor 5 contained 61 phages infecting the genus Staphylococcus and some Gram-negative bacteria. Factor 6 contained nine Lactobacillus phages and 36 Lactococcus phages, both of which could be associated with dairy food consumption or commensal lactic acid bacteria. We compared the factor scores between the MS and controls. We found that Factor 1 was lower, and Factor 5 was higher in MS than in controls, significantly (P < 0.01).

Conclusion: EFA can be a novel approach to classify gut viruses/phages regulating unique sets of bacteria in general virome analyses. EFA could also help in discovering gut phages that contribute to bacterial dysbiosis in MS.

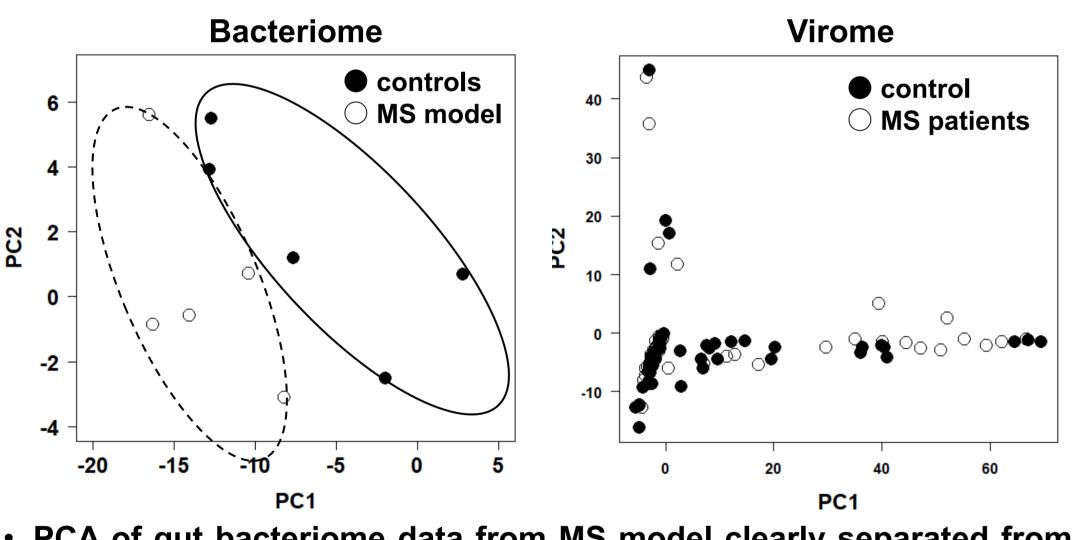
Introduction

Multiple sclerosis (MS) and microbiome



- Gut microbiota can activate systemic immune responses contributing to anti-microbial immunity.
- Uncontrolled excessive immune responses cause mediated tissue damage in remote organs.
 - →autoimmune diseases and/or MS
- Gut bacteriome modulation may suppress MS.
- Role of gut virome in MS has not been clarified.

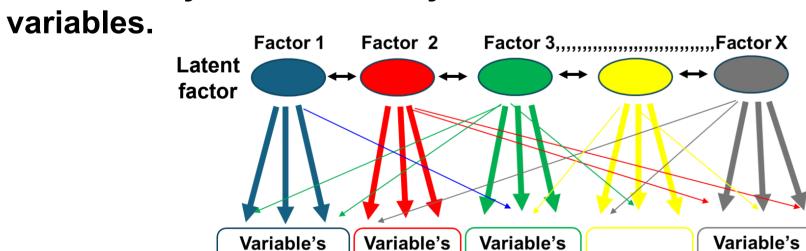
Principal component analysis (PCA) of bacteriome and virome data



- PCA of gut bacteriome data from MS model clearly separated from control mice (Omura et al., 2020).
- PCA of gut virome data did not distinguish between the MS patients and healthy controls.

Factor analysis

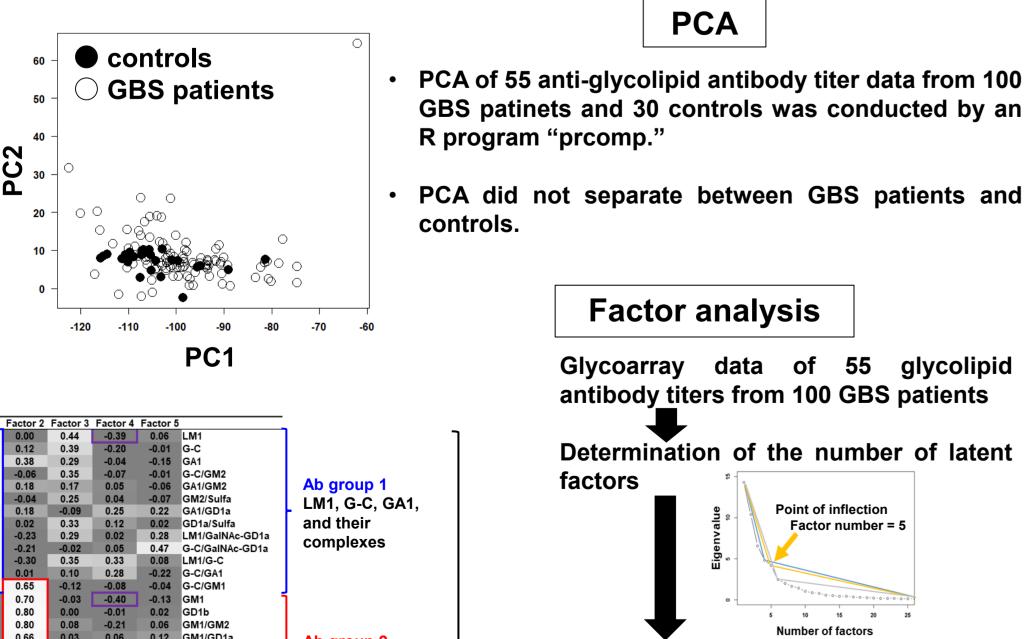
- Factor analysis has been used in the psychology field but not in biomedical field.
- Factor analysis can identify the latent factors/causes among the



group 2

PCA vs. factor analysis of glycoarray data from the patients with Guillain-Barre syndrome

group 3



Ab group 2 -55 glycolipids Ab group 3 0.15 GM2/LM1 GM2, GD1a, 0.36 GD1a/GalNAc-0 GQ1b, and their 0.11 GD1a/LM1 comlexes 0.23 GQ1b/GalNAc GD1b complexes, 0.22 GD1b/LM1 **GQ1b** complexes 0.11 GD1b/G-C and LM1 comlexes GalNAc-GD1a/GM2 GalNAx-GD1a -0.28 0.36 0.23 0.12 0.25 GD1a/GD1b

Five latent factors were identified and associated with distinct set of glycolipids located in peripheral nervous system.

55 glycolipid antibody titers from 100 GBS patients

group X

Determination of the number of latent Factor analysis of glycoarray data Glycolipid antibodies, whose factor loadings for factors were > 0.5,

were clustered into five groups Latent factor identification -0.48~0.66

GQ1b, and

LM1 complexes

GA1, and their GM1 complexes,

3/8 = 38% GM2, GD1a, GD1b complexes, GalNAc-GD1a IBD patients.

Aim

"To characterize the virome data of MS and other diseases by factor analysis"

Methods

The virome data were obtained as follows:

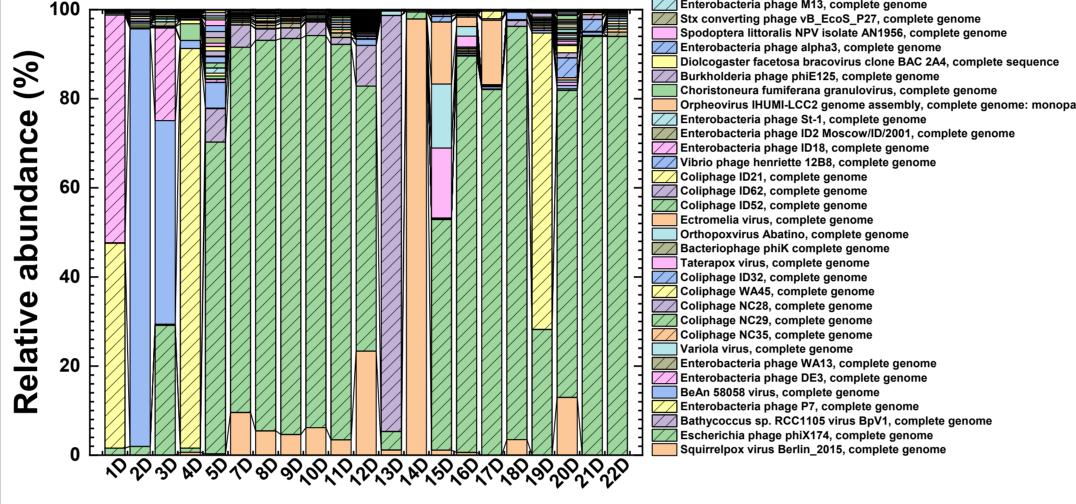
- Virome analysis of 22 lacrimal fluid samples using MinION device
- Virome data extracted from shotgun metagenome data (PRJNA391511) Fernandes et al., J Pediatr Gastroenterol Nutr, 2019) from 12 IBD patients and 12 healthy controls
- Virome data extracted from shotgun metagenome data (PRJNA1084182, Ghimire et al., Proc Natl Acad Sci USA, 2025) from 45 MS patients and 51 healthy controls

Virome data analysis

- ⇒ Mapping to RefSeq viral reference genome and counting read fragments ⇒ Factor analysis
 - → Determining factor number by scree plot
 - → Exploratory factor analysis by an R package "Psych"
 - → Grouping by factor loadings
 - → Latent factor identification

Results

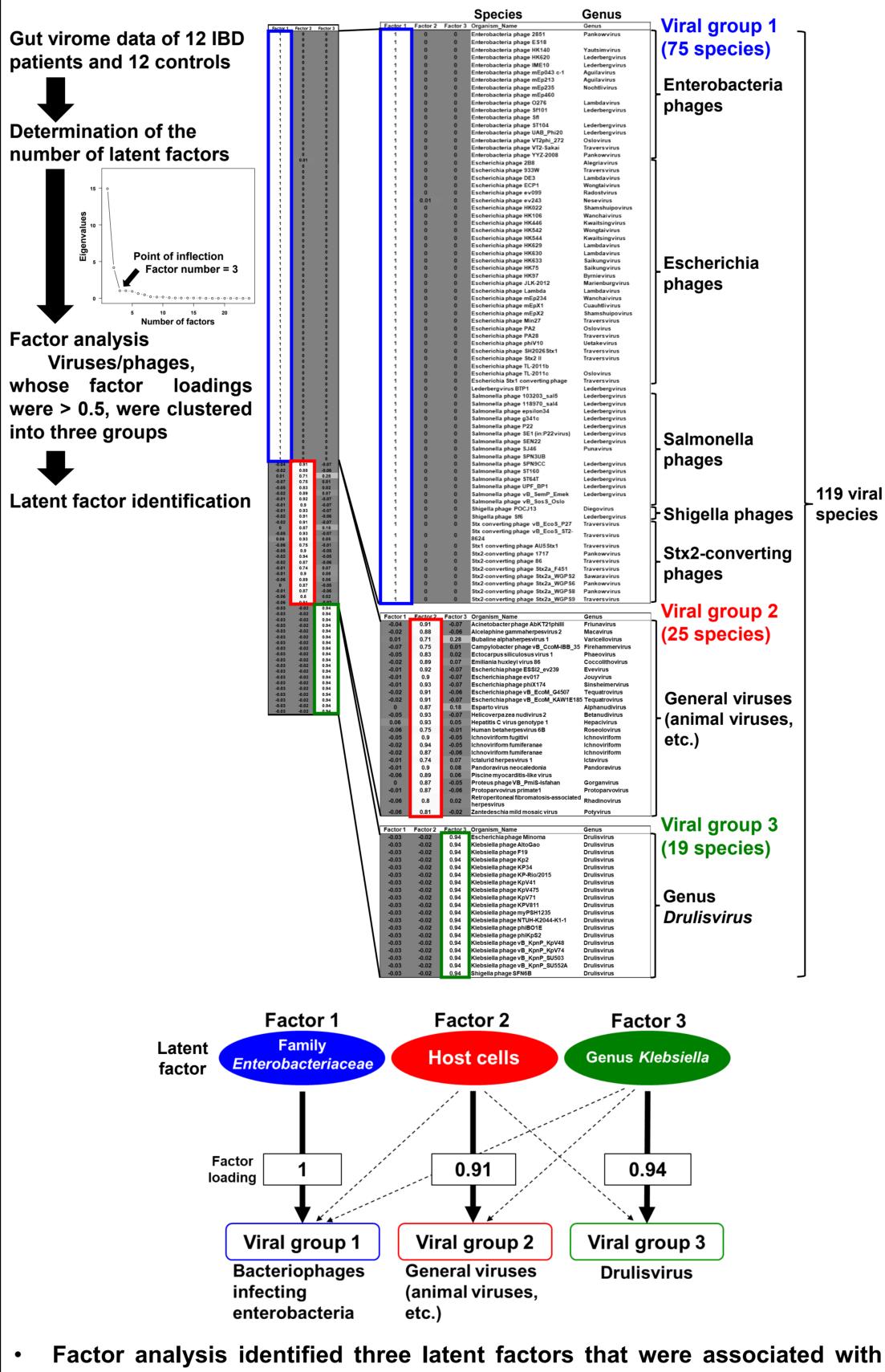
Virome is dominantly composed of bacteriophages (phages)



We determined the virome in human lacrimal fluid and found that virome was mostly composed of phages shown in hatched bars.

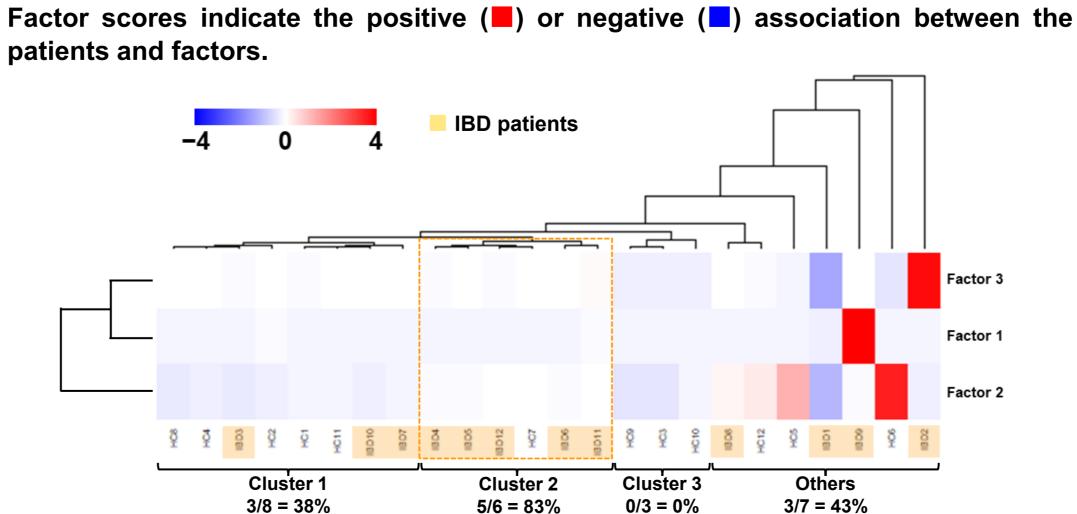
Factor analysis of gut virome in inflammatory bowel disease (IBD)

119 viral species (total 831,496 reads) were identified.



- Factor analysis identified three latent factors that were associated with bacteriophages (phages)/viruses infecting distinct hosts.
- Factor 1 appeared to be the phages infecting the Family
- Enterobacteriaceae. Factor 2 seemed to be general viruses, including animal viruses.
- Since Factor 3 was strongly associated with some IBD patients, bacteria in the genus Klebsiella, which has been linked to IBD etiologically (Federici et al., 2022), seemed pathogenic for some patients.

Dendrogram of IBD patients and controls based on the factor scores

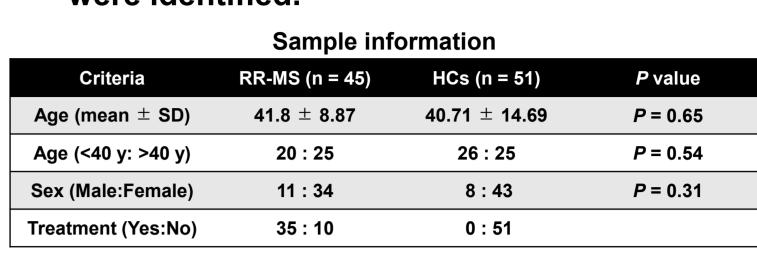


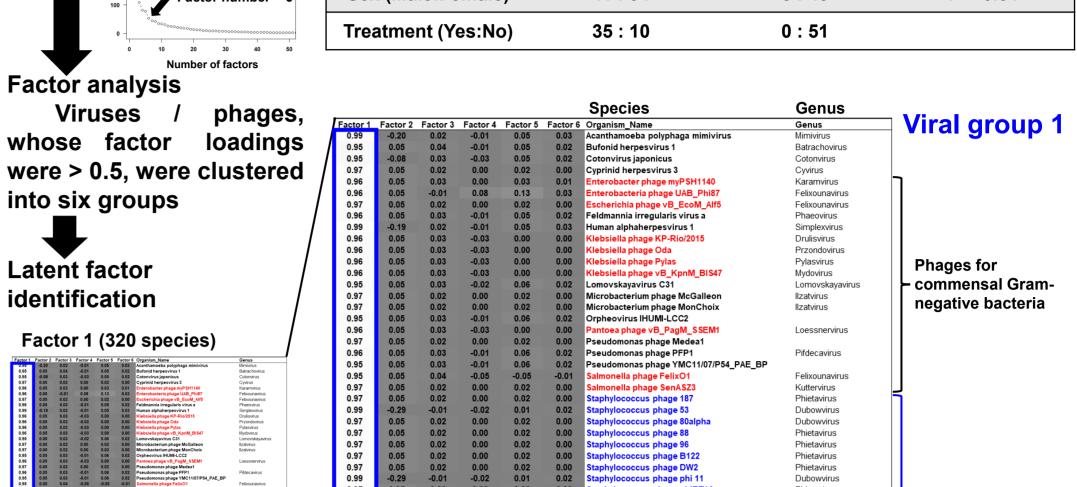
When a group composed of 3 or more people is defined as "Cluster," a total of 12 samples of IBD and controls (HCs) were divided into three clusters and others. Five of six (83%) of samples in Cluster 2 were from

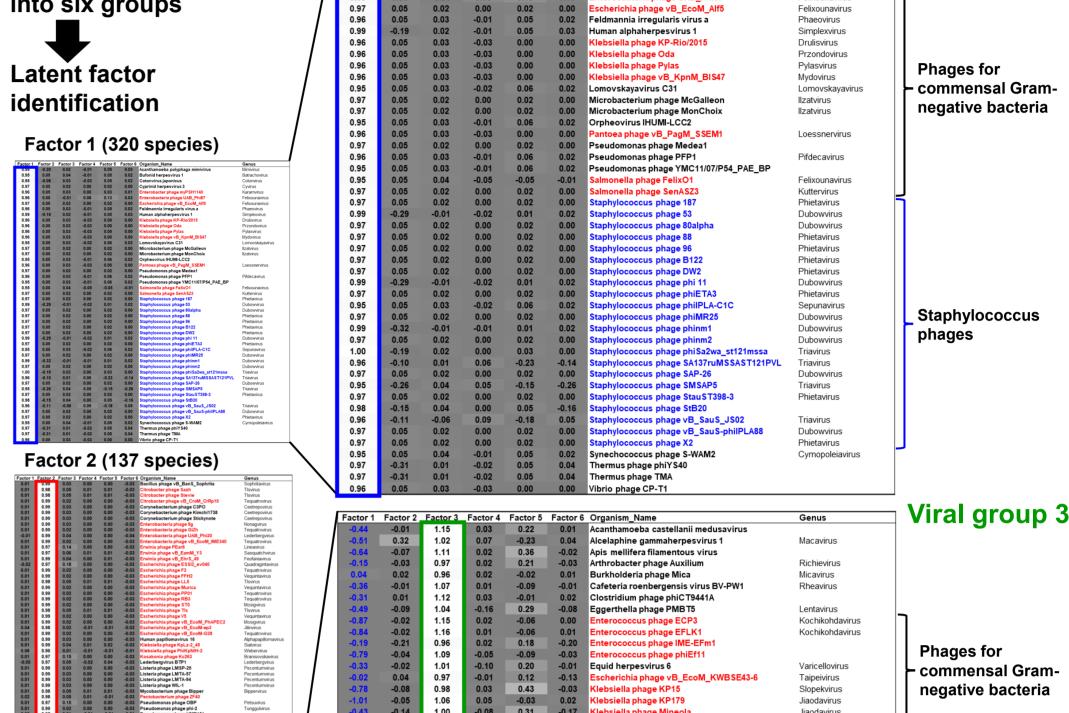
Factor analysis of gut virome in MS

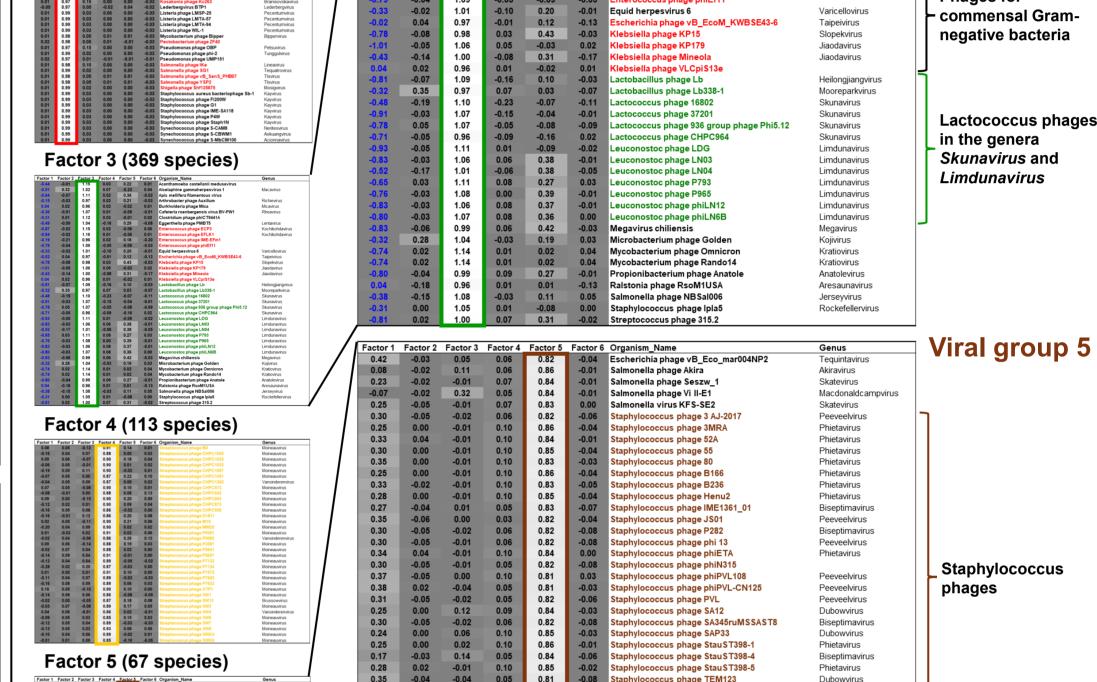
Gut virome data of 45 MS patients and 51 controls **Determination of the** number of latent factors

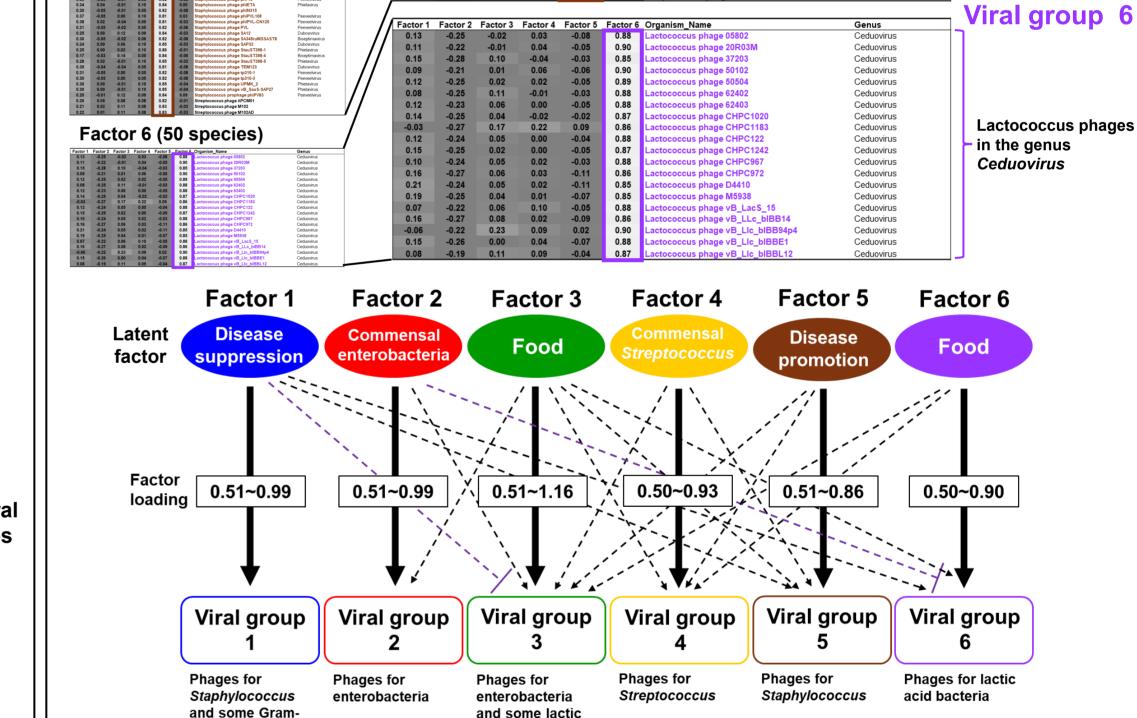
1,525 viral species (total 36,698,054 reads) were identified.











- Factor scores of factors 1 and 5 were associated with MS patients negatively and positively, which may indicate disease suppression and promotion, respectively (MS vs. factor 1 or 5, P < 0.01, chi-square test).
- Factors 2 and 4 appeared to be commensal bacteria; Factors 3 and 6 seemed to be food.
- Factors 1 and 2 might suppress viral groups 3 and 6, respectively.

Dendrogram of MS patients and controls based on the factor scores 0/12 = 0% 2/6 = 33% 9/9 = 100% 0/6 = 0%

When a group composed of six or more people is defined as "Cluster," a total of 96 samples of MS and HCs were divided into eight clusters and others. Several clusters included a high proportion of MS patients.

Clusters 4, 7, and 8 contained no MS patients

Conclusions

- PCA didn't distinguish the virome between MS patients and controls.
- Factor analysis could identify the latent factors that were associated with unique sets of bacteriophages.
 - Klebsiella phages in genus *Drulisvirus*, that might be associated with disease pathogenesis. In MS, bacteriophages in Factors 1 and 5 might contribute to the

• In IBD, factor analysis identified viral group 3, which contains

- disease conditions of MS.
- A dendrogram of factor scores may result in the identification of distinct subpopulations in IBD and MS.

References

- Omura S, et al., (2020). Bioinformatics analysis of gut microbiota and CNS transcriptome in virus-induced acute myelitis and chronic inflammatory demyelination; potential association of distinct bacteria with CNS IgA
- upregulation. *Front Immunol*, 11:1138. doi: 10.3389/fimmu.2020.01138. ● Omura S, et al. (2022). Exploratory factor analysis determines latent **□** □ □ □ □ □ factors in Guillain-Barré syndrome. Scientific Reports, 12:21837. doi: 10.1038/s41598-022-26422-5.
- Ahmad I, Omura S, et al. (2025). The safety of *Alcaligenes* lipid A in an immune-mediated disease model associated with IgA, Th17 cells, and gut microbiota. Int J Mol Sci. In review.

This study has no conflict of interest.