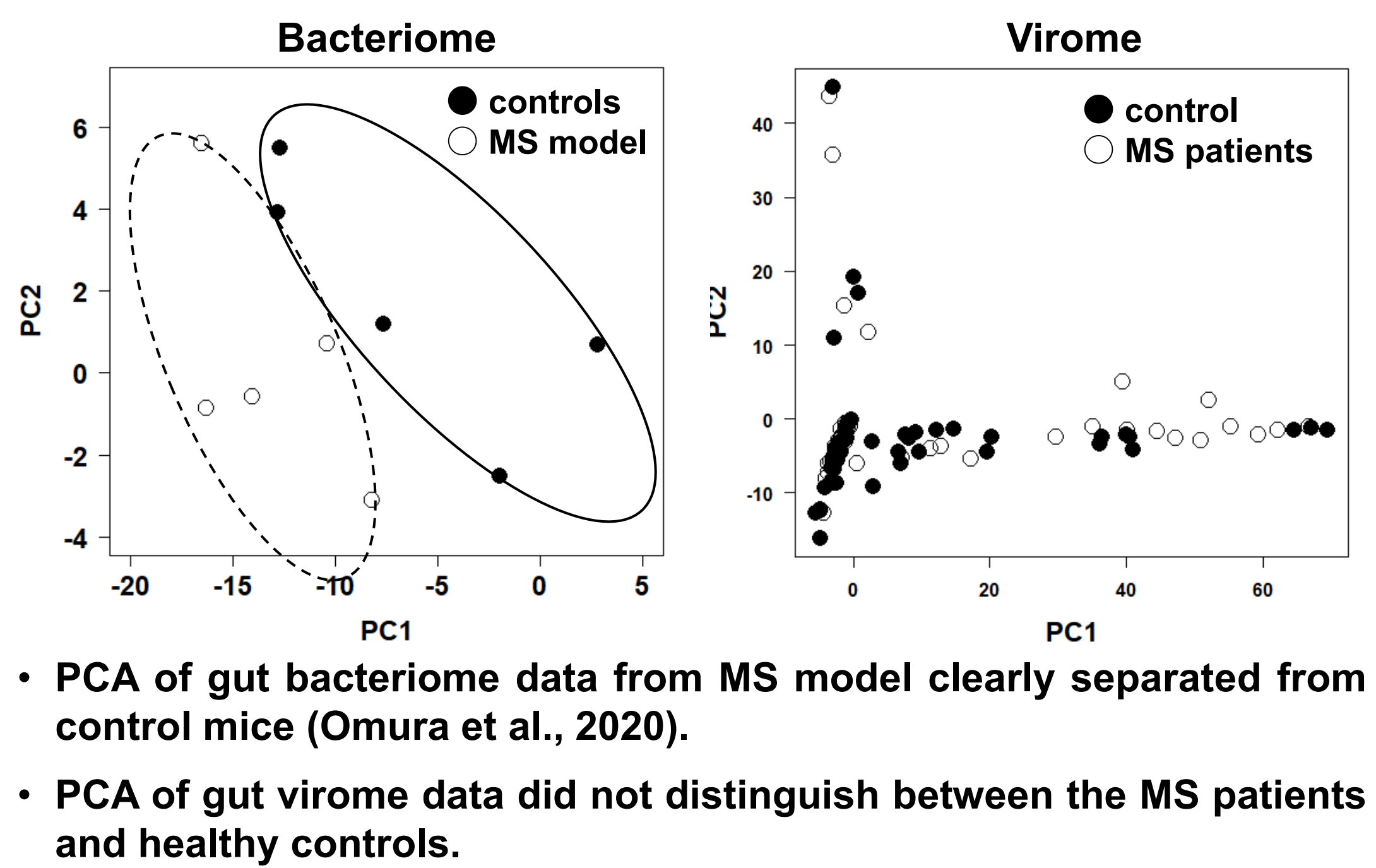


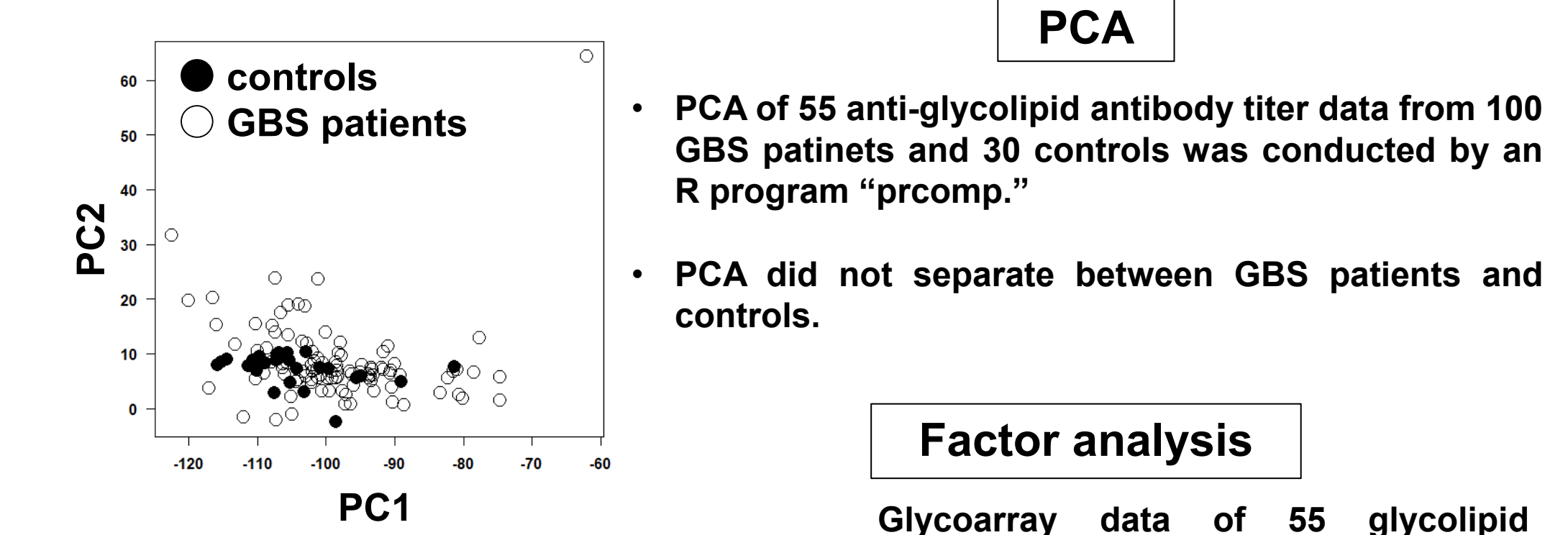
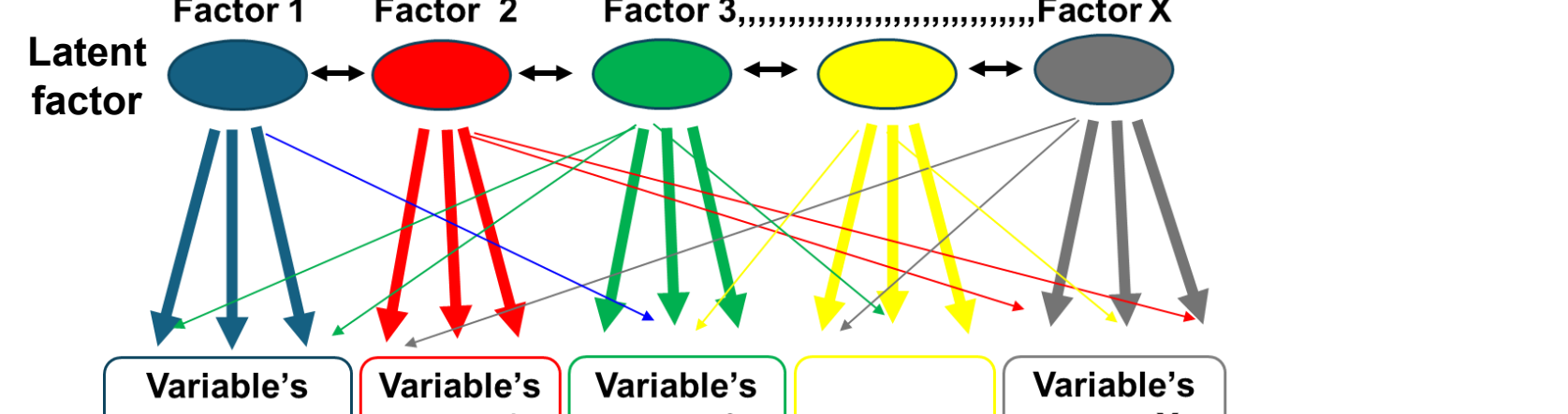
Department of Microbiology, Kindai University Faculty of Medicine, Osaka, Japan Ikuo Tsunoda, M.D., Ph.D.
itsunoda@hotmail.com

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.

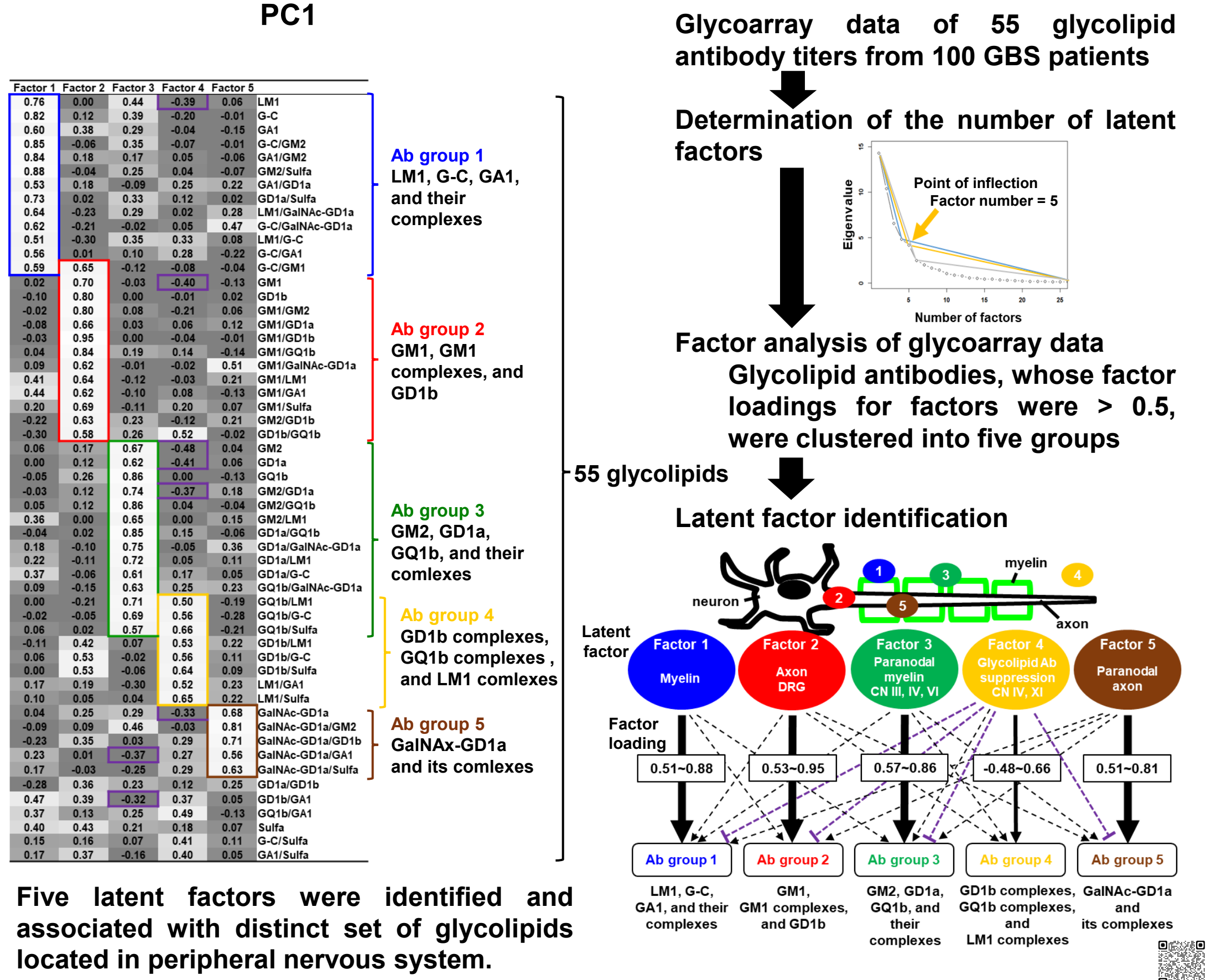
- Gut microbiota can activate systemic immune responses contributing to anti-microbial immunity.
- Uncontrolled excessive immune responses cause immune-mediated tissue damage in remote organs.
→ autoimmune diseases and/or MS
- Gut bacteriome modulation may suppress MS.
- Role of gut virome in MS has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



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



- PCA of 55 anti-glycolipid antibody titer data from 100 GBS patients and 30 controls was conducted by an R program “prcomp.”
- PCA did not separate between GBS patients and controls.

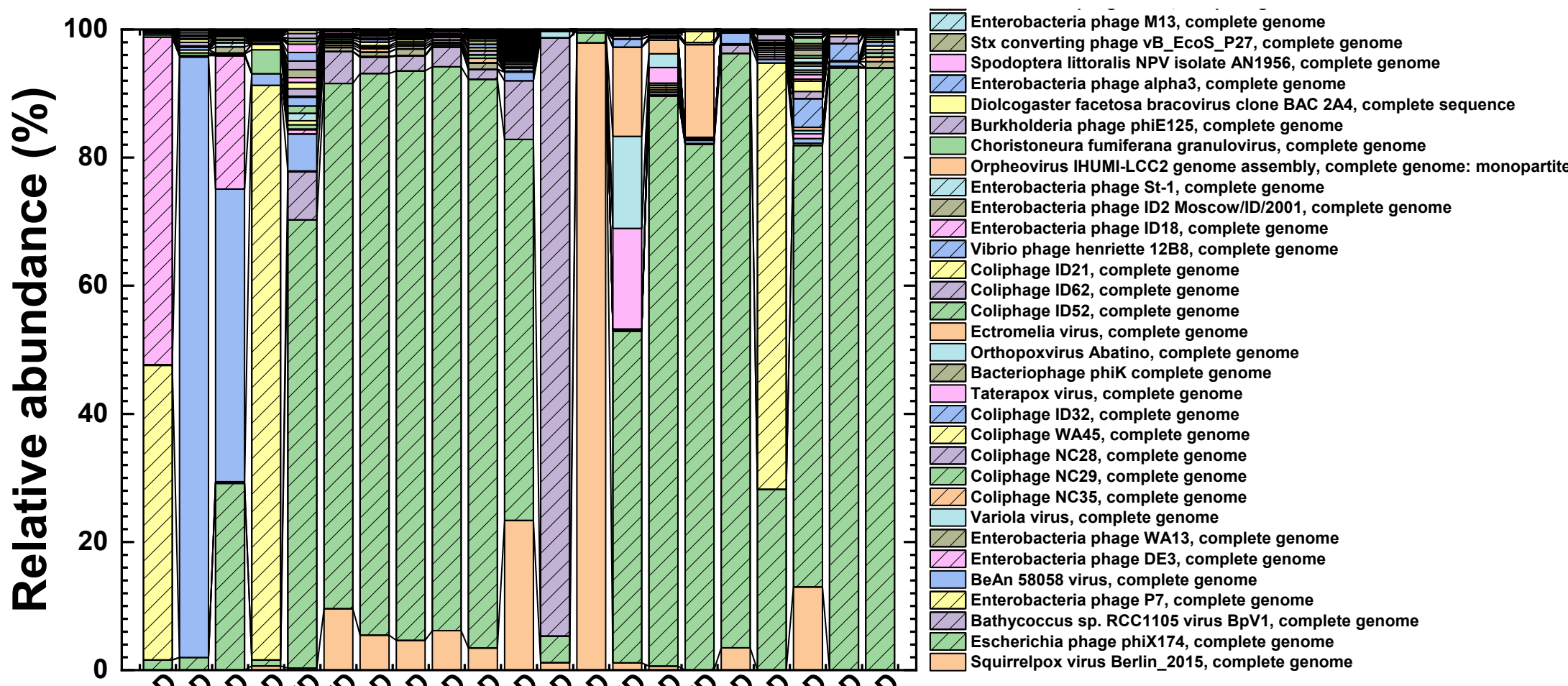


“To characterize the virome data of MS and other diseases by factor analysis”

The virome data were obtained as follows:

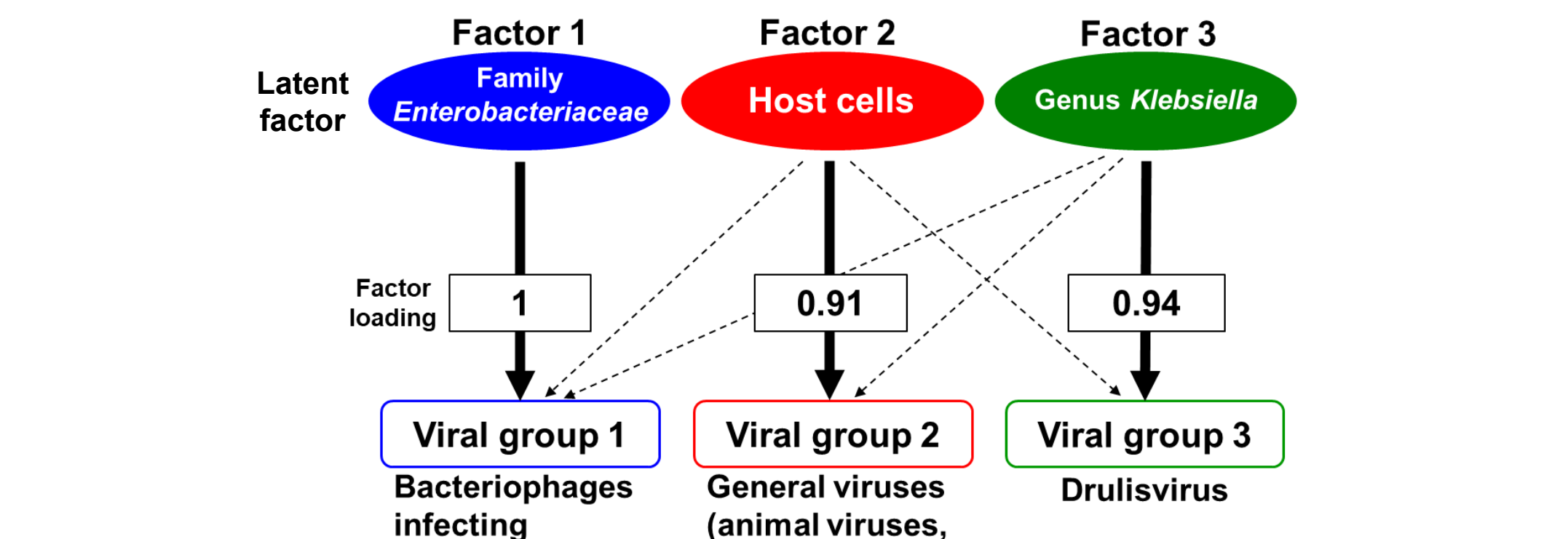
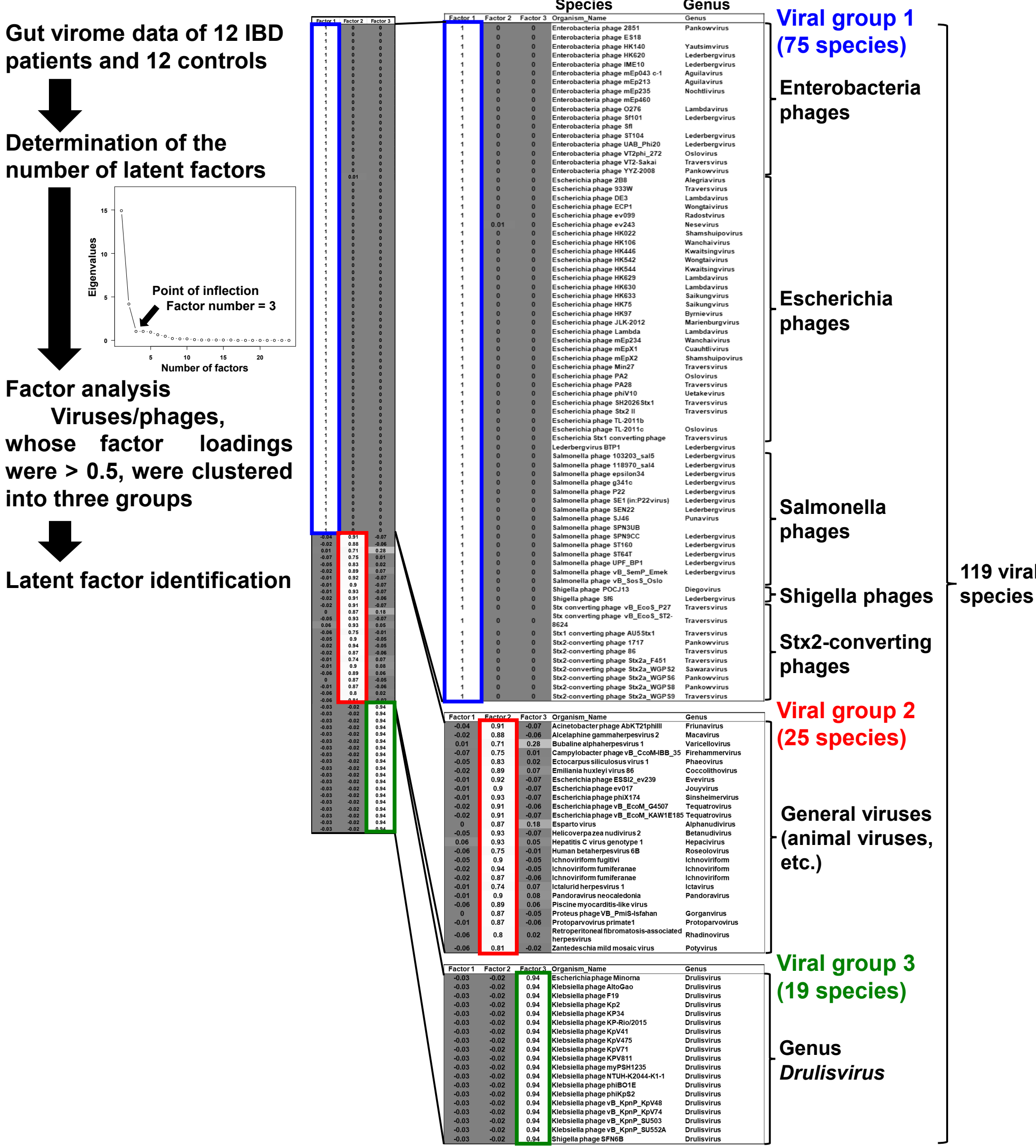
- Virome analysis of 22 lacrimal fluid samples using MinION device
- Virome data extracted from shotgun metagenome data (PRJNA391511 Fernandes et al., *J Pediatr Gastroenterol Nutr*, 2019) from 12 IBD patients and 12 healthy controls
- Virome data extracted from shotgun metagenome data (PRJNA1084182 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



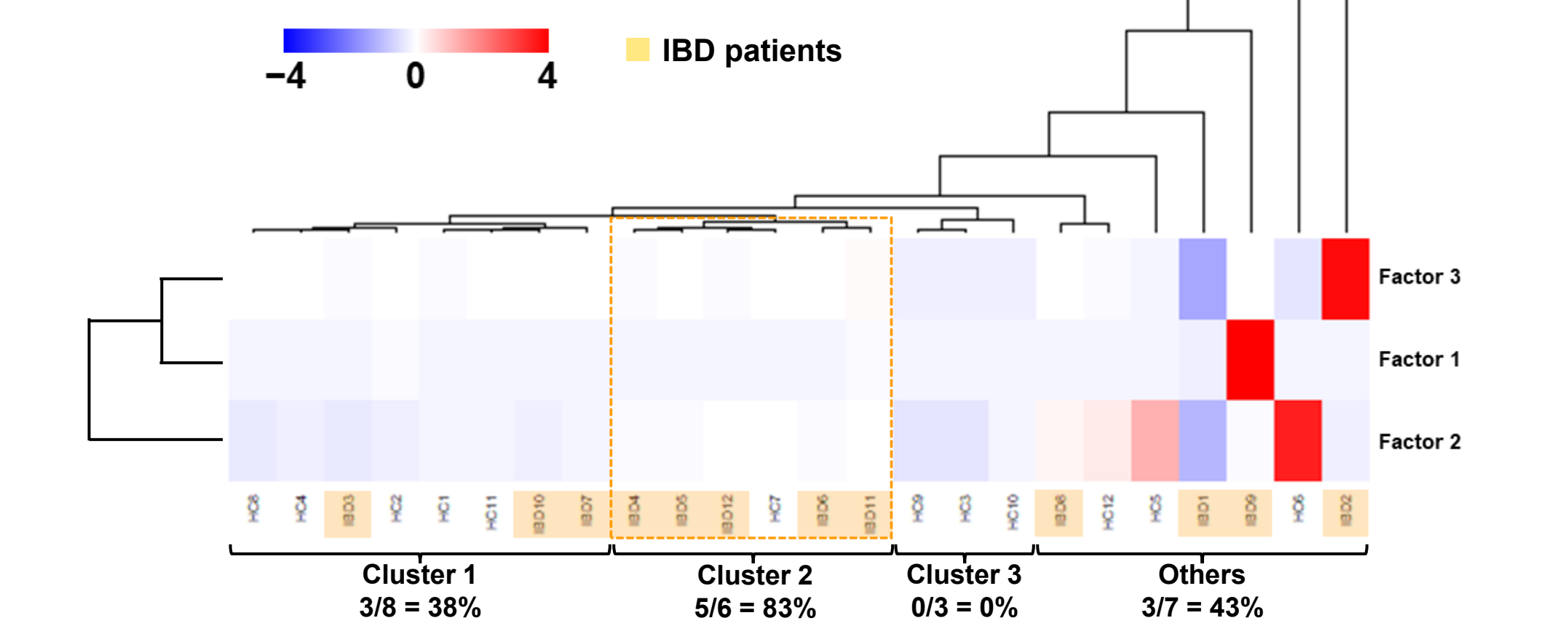
We determined the virome in human lacrimal fluid and found that virome was mostly composed of phages shown in hatched bars.

- **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.

Factor scores indicate the positive (■) or negative (■) association between the patients and factors.



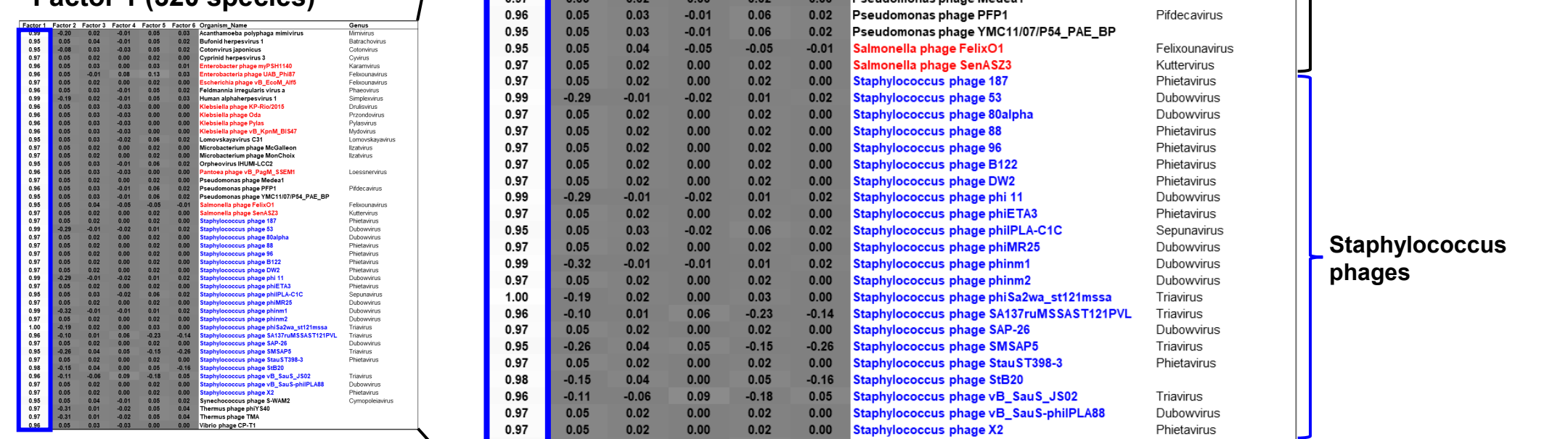
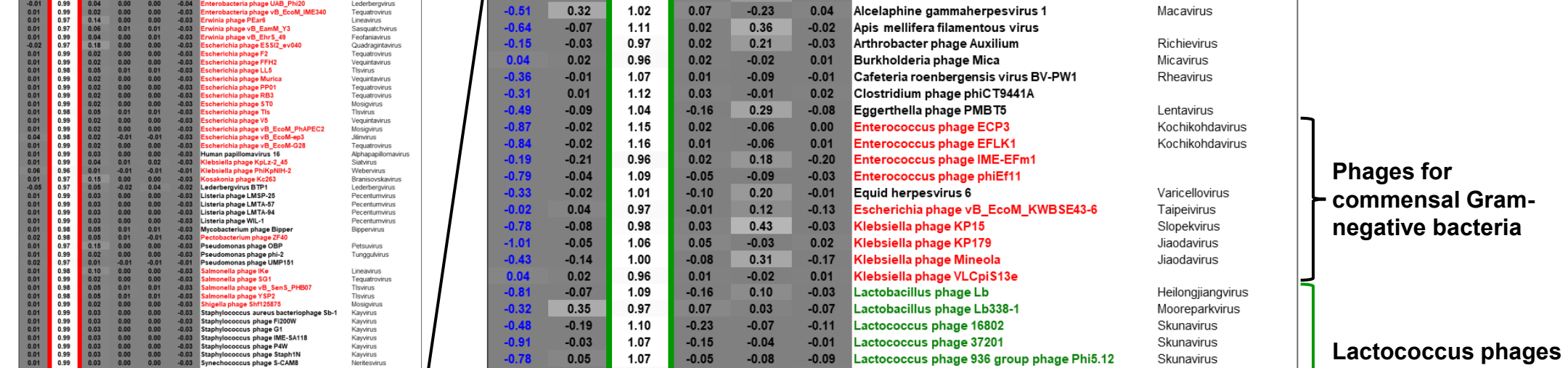
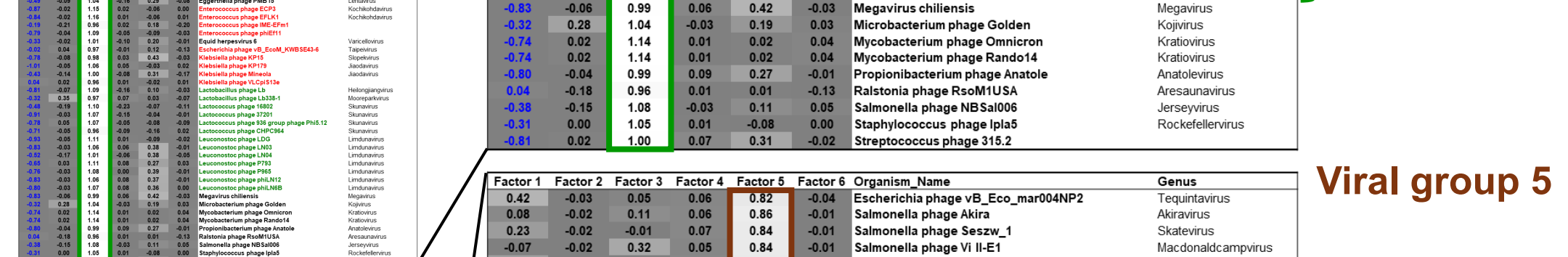
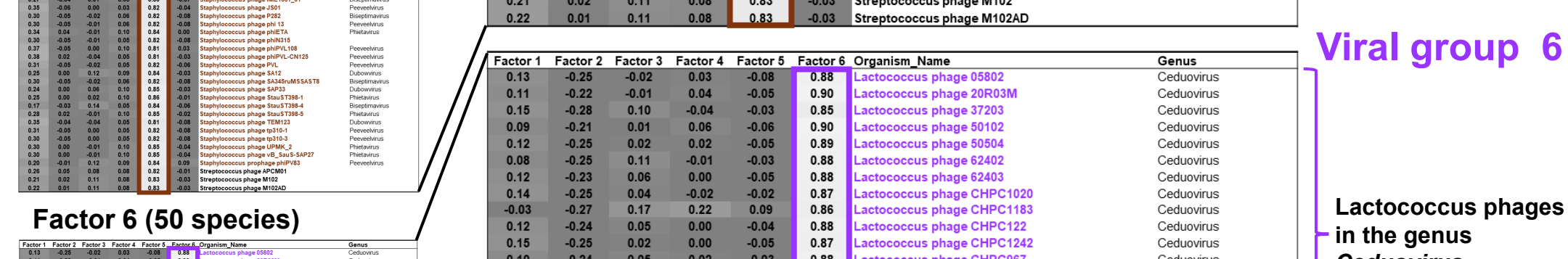
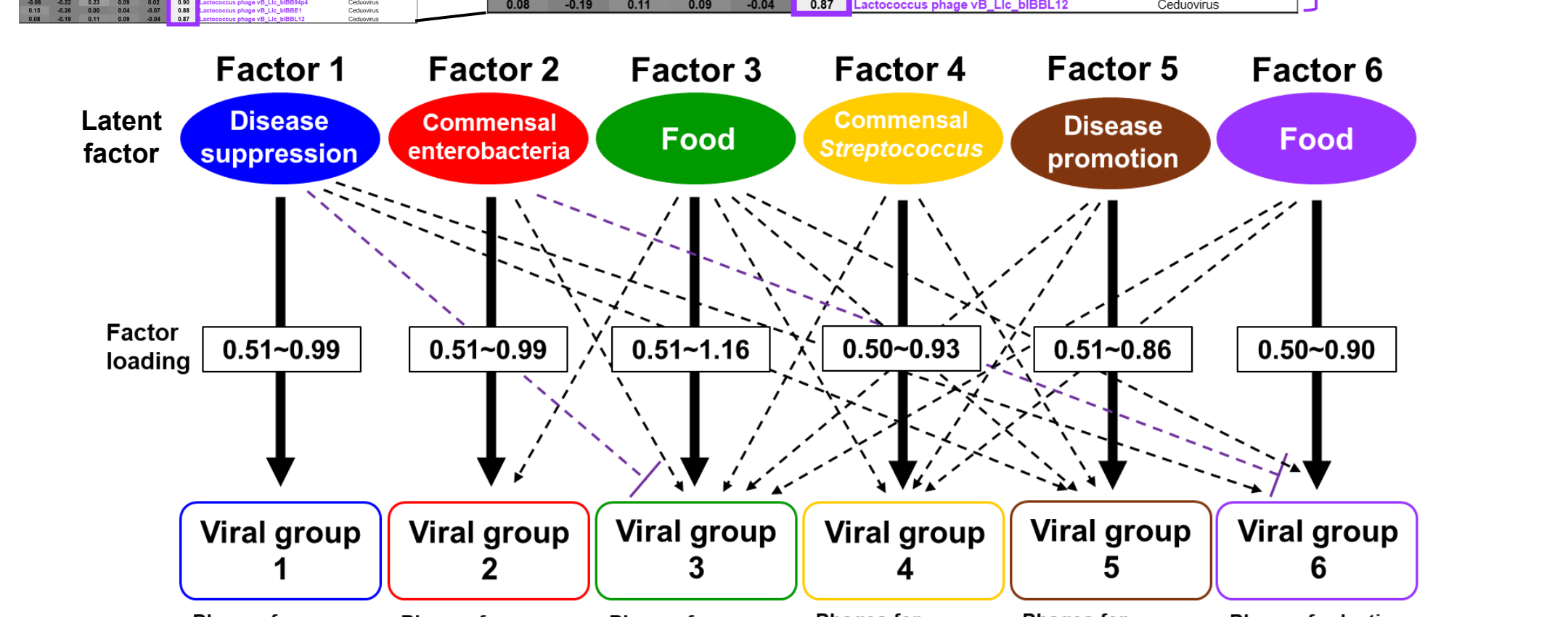
- When a group composed of 3 or more people is defined as “Cluster,” a total of 12 samples of IBD and controls (HCs) were divided into three clusters and others. Five of six (83%) of samples in Cluster 2 were from IBD patients.

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

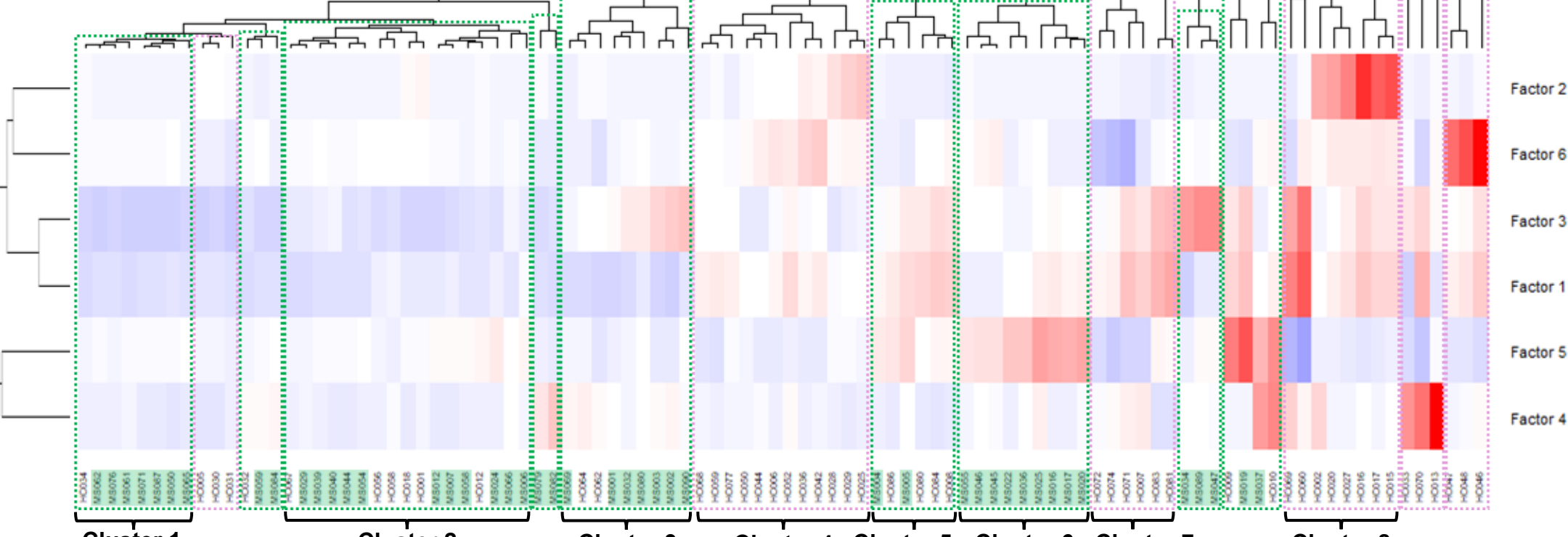
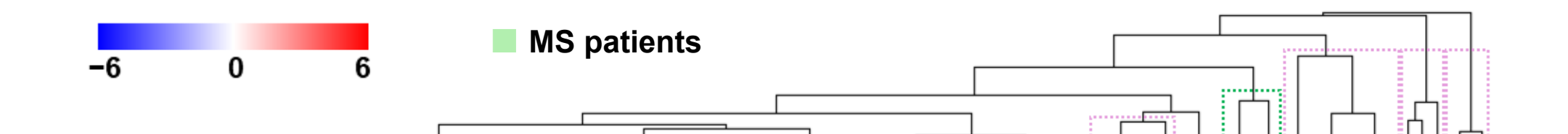
Sample information			
Criteria	RR-MS (n = 45)	HCS (n = 51)	P value
Age (mean $\pm$ SD)	41.8 $\pm$ 8.87	40.71 $\pm$ 14.69	$P = 0.65$
Age (<40 y: >40 y)	20 : 25	26 : 25	$P = 0.54$
Sex (Male:Female)	11 : 34	8 : 43	$P = 0.31$
Treatment (Yes:No)	35 : 10	0 : 51	

[illegible]

Accession	Length	Score	E-value	Description	Organism		
0.97	0.05	0.02	0.00	0.02	0.00	Microbacterium phage MonChek	Escherichia
0.96	0.05	0.03	-0.01	0.04	0.02	Orypheovirus IHUM-LCC2	
0.96	0.05	0.03	-0.03	0.00	0.00	Parvexa phage vB_ParM1_S16M1	Leishmanium

[illegible][illegible][illegible][illegible][illegible]

- 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.



- 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

- 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.
 - In IBD, factor analysis identified viral group 3, which contains *Klebsiella* phages in genus *Drulisvirus*, that might be associated with disease pathogenesis.
 - In MS, bacteriophages in Factors 1 and 5 might contribute to the disease conditions of MS.
 - A dendrogram of factor scores may result in the identification of distinct subpopulations in IBD and MS.

- 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.