Exploratory Factor Analysis Determines Latent Factors Associated with Distinct Sets of Anti-Glycolipid Antibodies in Guillain-Barré Syndrome

KINDAI UNIVERSITY
KANSAI UNIVERSITY
FUKUSHIMA MEDICAL UNIVERSITY

Seiichi Omura[1], Kazuaki Shimizu[3], Miyuki Morikawa[2], Motoi Kuwahara[2], Takashi Kawamura[4], Tsutomu Mori[4], Susumu Kusunoki[2], Ikuo Tsunoda[1]

[1]Departments of Microbiology and [2]Neurology, Kindai University Faculty of Medicine, Osaka
[3]Department of Psychology, Faculty of Sociology, Kansai University, Osaka
[4]Department of human Lifesciences, Fukushima Medical University School of Nursing, Fukushima

Seiichi Omura, PhD
omura.s@hotmail.com
http://tsunodalaboratory.web.fc2.com/

Abstract

Exploratory factor analysis (EFA) has been developed as a powerful statistical procedure in psychological research. EFA's purpose is to identify the nature and number of latent constructs (=factors) underlying a set of observed variables. Since the research goal of EFA is to determine what causes the observed responses, EFA is ideal for hypothesis-based studies, such as identifying the latent factors or causes of diseases. However, the application of EFA in the biomedical field is generally limited. Guillain-Barré syndrome (GBS) is peripheral neuropathy, in which the presence of antibodies to glycolipids are often associated with clinical signs. Although the precise mechanism for generation of anti-glycolipid antibodies is unclear, infections with microbes whose molecular structures mimic glycolipids have been proposed to induce antibodies to microbes as well as peripheral nerves expressing the glycolipids. We hypothesized that latent factors, such as distinct microbes, could induce different sets of anti-glycolipid antibodies in subsets of GBS patients. Combinatorial glycoarray allowed as to quantify serum antibody titers against 10 glycolipids and 45 combinations of two different glycolipids. Using antibody data from 47 samples, we conducted EFA with an R package "psych". We used the Scree plot for determination of the number of factors, a principal factor method for extraction of factors, and a promax method for factor rotation. We extracted five factors, each of which was comprised of the distinct set of antibodies to glycolipids. Future application of EFA of glycoarray data into more comprehensive data of GBS patients may give insight into the potential factors (or causes), which induce distinct antibodies against a set of glycolipids that are associated with specific clinical signs.
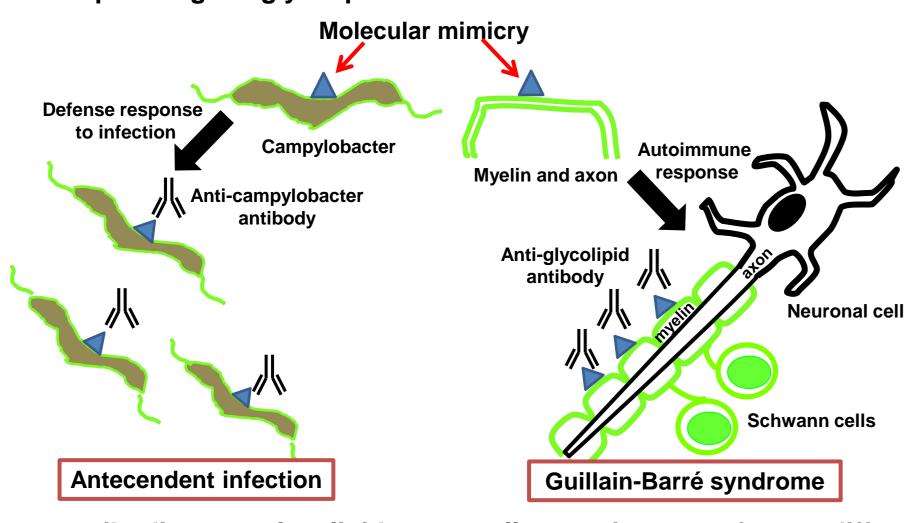
Introduction

What is exploratory factor analysis (EFA)?
- EFA has been developed as a powerful statistical procedure in psychological research.
- The purpose is to identify the nature and number of latent constructs (= factors) underlying a set of observed variables.
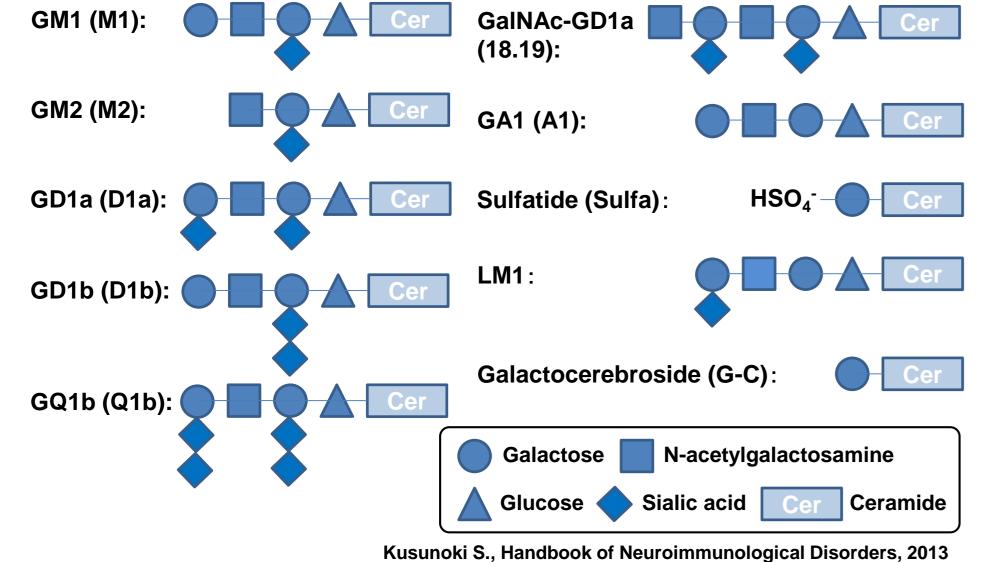- EFA is used when the research goal is to determine what causes the observed responses.

What is Guillain-Barré syndrome (GBS)?
- An acute immune-mediated peripheral neuropathy with symmetrical weakness of the limbs, and areflexia.
- The precise mechanism for generation of anti-glycolipid antibodies is unclear.
- Infections with microbes whose molecular structures mimic glycolipids have been proposed to induce antibodies to microbes as well as peripheral nerves expressing the glycolipids.

- Since antibodies to glycolipids as well as mixtures of two different glycolipids have been detected in sera from GBS patients, some anti-glycolipid antibodies can be useful diagnostic markers.
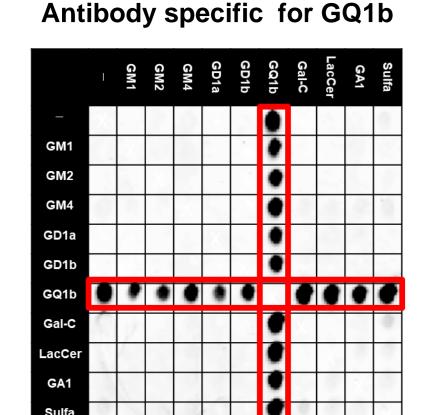- Glycolipids related to GBS:

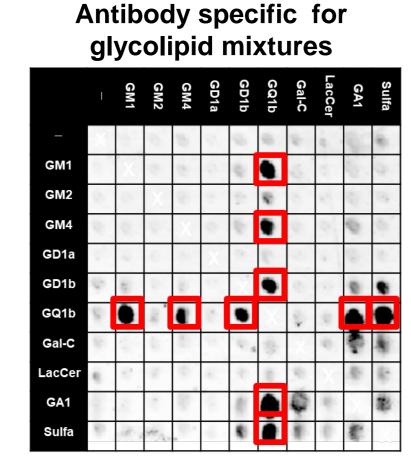Kusunoki S., Handbook of Neuroimmunological Disorders, 2013

What is glycoarray?
- A method for detecting a set of glycolipid antibodies simultaneously, using membranes spotted with individual glycolipids and/or mixtures of two different glycolipids.
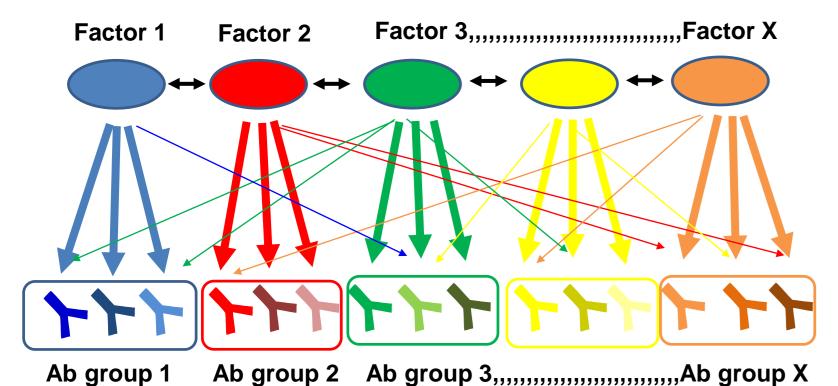- Example:

Hypothesis

"Factor 1 to X induce groups of distinct glycolipid antibodies"

Antibodies (Abs) to 10 glycolipids and 45 glycolipid complexes (G1 to G55-Ab)
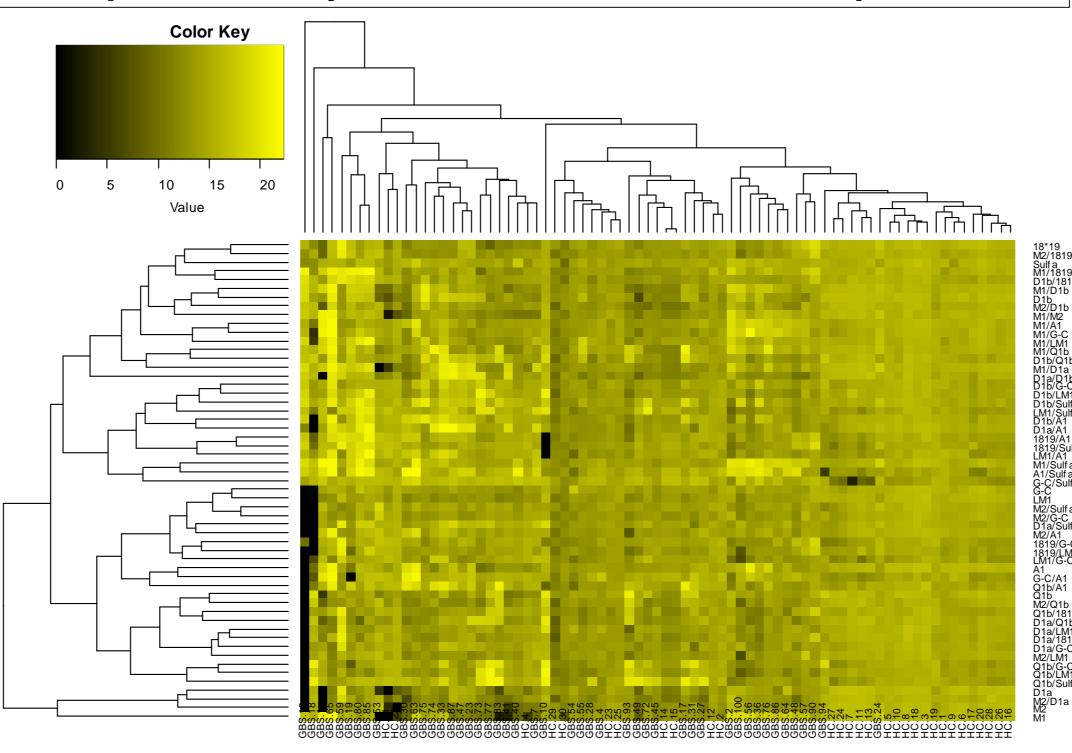
Materials & Methods

Sample preparation: Sera were obtained from 100 patients with GBS during the acute or relapsing phase of neuropathies as well as 30 healthy controls (HC) in Kindai University Hospital, Osaka, Japan, and other institutions in Japan (Morikawa et al., 2016).

Combinatorial glycoarray: We detected serum antibodies against 10 glycolipids and 45 combinations of two different glycolipids spotted onto Immobilon-FL polyvinylidene difluoride (PVDF) membrane (Merk Millipore) by Alexa 555 anti-human IgG antibodies (Thermo Fisher Scientific). Antibody titers were expressed in fluorescent intensities quantified by Image Quent TL software (GE healthcare), while intensities greater than the mean plus three standard deviations (SD) for 30 HC samples were considered positive. Among 100 GBS patients, 47 patients showed anti-glycolipid antibody positive.
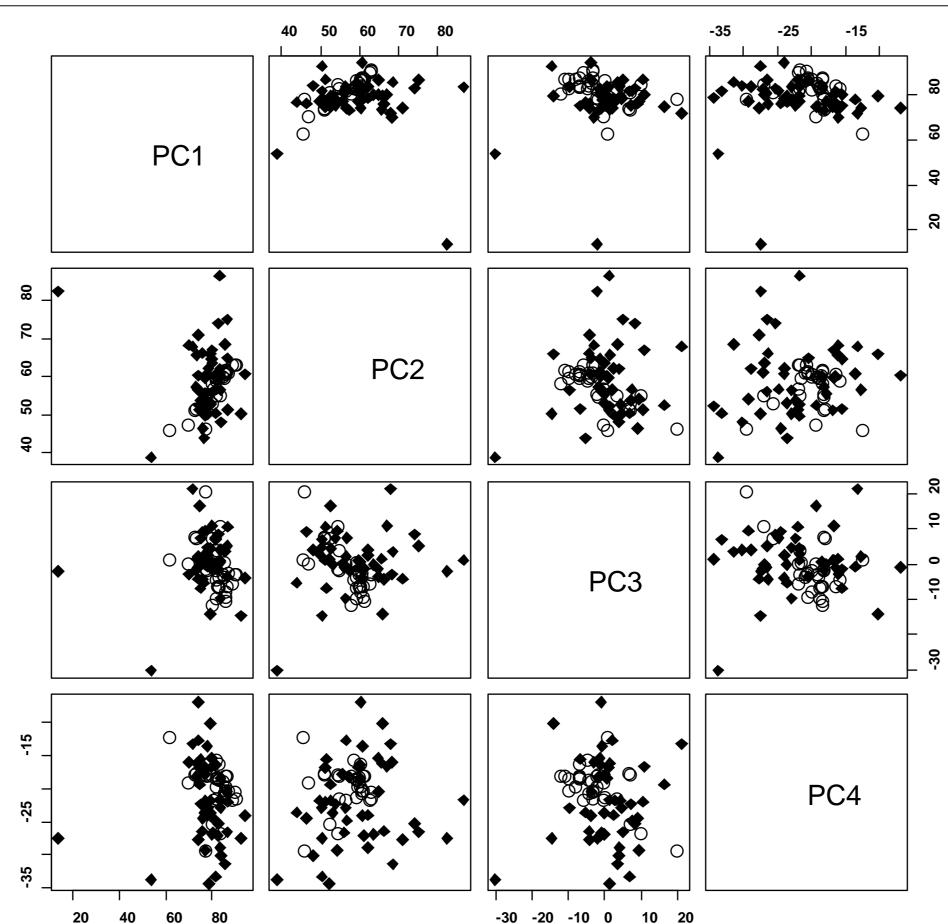
Data analyses: Data were changed to logarithm ($\log_2$). Hierarchical clustering and principal component analysis (PCA) were conducted using R version 3.3.2 and the functions "genefilter", "gplots", and "prcomp". EFA was conducted on glycoarray data, using an R package "psych". We used the Scree plot for determination of the number of factors, a least-square method for extraction of factors, and a promax method for factor rotation.

Results

Hierarchical clustering of serum glycoarray data does not separate clearly between 30 HC and 47 GBS patients
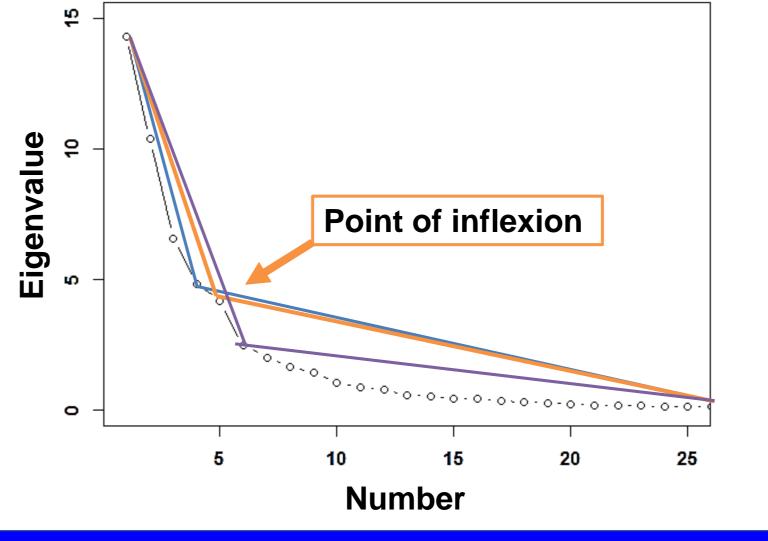

PCA of serum glycoarray data does not separate between HC (○) and GBS patients (◆)

Factor analysis extracts 5 factors from serum glycoarray data of GBS patients and associates with clinical data

A. Scree plot determines the number of factors

Point of inflexion

B. Factor loading separates the antibodies into 5 groups

	MR1	MR2	MR3	MR4	MR5	h2	u2	com
LM1	0.82	0.33	0.13	-0.16	0.21	0.83	0.175	1.6
G-C	0.87	0.25	0.19	-0.14	0.06	0.81	0.188	1.3
A1	0.75	0.18	0.5	-0.16	-0.22	0.75	0.249	2.2
Sulfa	0.65	0.04	0.47	-0.06	-0.12	0.55	0.454	1.9
M2/LM1	0.55	0.53	-0.02	-0.15	0.16	0.65	0.354	2.3
M2/G-C	0.69	0.18	0.05	-0.13	0	0.55	0.447	1.1
M2/A1	0.9	0	0.23	0.04	-0.1	0.9	0.098	1.1
M2/Sulfa	0.92	0.07	0.04	-0.09	-0.1	0.86	0.14	1.1
D1a/G-C	0.59	0.56	-0.09	0.08	0.05	0.72	0.283	2.1
D1a/A1	0.66	-0.24	0.47	0.4	0.2	0.79	0.212	2.2
D1a/Sulfa	0.86	-0.2	-0.07	0.13	0.05	0.83	0.166	1.2
D1b/A1	0.51	-0.48	0.12	0.47	-0.05	0.83	0.165	3.1
D1b/A1	0.63	0.1	-0.04	0.25	-0.29	0.57	0.434	1.8
18.19/LM1	0.87	0.17	-0.04	-0.08	0.27	0.86	0.135	1.3
18.19/G-C	0.78	-0.3	-0.18	-0.13	0.36	0.84	0.161	1.9
LM1/G-C	0.76	0.2	-0.26	0.05	-0.03	0.78	0.223	1.4
G-C/A1	0.73	0.01	0.18	-0.15	-0.4	0.68	0.318	1.8
G-C/Sulfa	0.37	-0.24	-0.17	0.18	-0.4	0.41	0.594	3.4
M2	0.18	0.77	0.23	-0.04	0.27	0.66	0.34	1.6
D1a	0.12	0.72	0.14	0.05	0.29	0.57	0.433	1.5
Q1b	0.09	0.89	0.02	0.1	-0.1	0.8	0.202	1.1
M2/D1a	0.15	0.83	0.07	0.06	0	0.79	0.209	1.6
M2/Q1b	0.22	0.8	-0.04	-0.03	-0.06	0.74	0.263	1.2
D1a/Q1b	0.2	0.82	-0.2	0.22	-0.03	0.84	0.158	1.4
D1a/18.19	0.47	0.65	-0.1	-0.15	0.36	0.81	0.19	2.7
D1a/LM1	0.67	-0.16	-0.09	0.14	0.75	0.247	2
Q1b/18.19	0.49	0.62	-0.18	0.01	0.12	0.73	0.268	2.2
Q1b/LM1	0.55	0.6	-0.32	0.17	-0.34	0.81	0.188	2.8
Q1b/G-C	0.22	0.63	-0.21	0.15	-0.44	0.82	0.179	2.5
Q1b/Sulfa	0.27	0.47	-0.33	0.18	-0.46	0.79	0.206	3.8
M1	-0.06	0.12	0.83	-0.07	0.08	0.7	0.295	1.2
M1/M2	-0.04	0.08	0.76	0.04	0.13	0.71	0.291	1.1
M1/D1b	-0.1	0.03	0.78	0.43	0.06	0.9	0.103	1.6
M1/Q1b	-0.03	0.2	0.66	0.29	-0.19	0.5	0.502	1.8
M1/LM1	0.35	-0.28	0.5	0.23	0.29	0.74	0.264	3.7
M1/G-C	0.48	-0.23	0.76	-0.08	-0.02	0.81	0.187	1.9
M1/A1	0.43	-0.24	0.83	-0.13	-0.19	0.88	0.116	1.9
M1/Sulfa	0.12	-0.27	0.76	-0.01	0.02	0.74	0.264	1.3
D1b	-0.14	0.07	0.47	0.69	0.08	0.8	0.204	1.9
M1/D1a	-0.03	0.04	0.33	0.5	0.19	0.47	0.531	2.1
M2/D1b	-0.07	0.29	0.33	0.5	0.36	0.65	0.345	4
D1a/D1b	-0.19	0.27	-0.13	0.7	0.33	0.67	0.327	2
D1b/Q1b	-0.26	0.35	0.07	0.84	-0.16	0.76	0.236	1.5
D1b/LM1	-0.03	0.03	-0.09	0.81	0.11	0.68	0.315	1.1
D1b/G-C	0.05	-0.11	0.2	0.79	-0.12	0.72	0.276	1.2
D1b/Sulfa	0.26	-0.22	0.02	0.8	-0.14	0.74	0.262	1.2
LM1/Sulfa	0.25	-0.22	-0.5	0.46	0.01	0.57	0.434	2.9
18.19	0.04	0.25	0.11	-0.04	0.87	0.8	0.201	1.2
M1/18.19	0.1	-0.16	0.42	0.17	0.6	0.79	0.21	2.2
M2/18.19	-0.04	0.35	-0.18	-0.01	0.87	0.83	0.166	1.4
D1b/18.19	-0.05	0.05	0.04	0.38	0.74	0.82	0.178	1.6
18.19/Sulfa	0.23	-0.61	-0.31	-0.06	0.52	0.71	0.293	2.8
18.19/A1	0.36	-0.62	-0.16	-0.02	0.45	0.73	0.266	2.6
LM1/A1	0.26	-0.58	-0.09	0.25	0.05	0.52	0.483	1.9
A1/Sulfa	0.2	-0.41	-0.33	0.07	0.21	0.37	0.629	2.5

MR1-5 indicates the factors. The "h2" indicates commonalities, "u2" indicates uniquenesses, and "com" indicates complexity of factor loadings for variables. Factor 1 is composed of LM1, G-C, A1, Sulfa and their complexes. Factor 2 is composed of M2, D1a, Q1b and their complexes. Factors 3, 4 and 5 are composed of M1 complexes, D1b complexes and 18.19 complexes, respectively.

C. Pattern of factor scores associates factor 2 with cranial nerve symptoms

Patient	MR1	MR2	MR3	MR4	MR5	age	sex	antecedent infection	disease peak	cranial nerve
7	0.64	-0.27	-0.91	0.00	-0.06	66	M	Yes, respiratory	Day 6	No
27	0.23	0.10	-0.54	-0.53	-0.72	20	F	Yes, respiratory	Day 4	III, VI, VII, IX, X, X I
30	0.64	-1.07	-0.87	-1.14	-1.34	63	M	Yes, digestive	Day 4	No
40	0.24	-0.45	-0.79	-0.63	-0.37	55	M	Yes, respirator	Day 28	No
49	0.12	-0.19	-1.50	-0.92	-0.96	53	M	Yes, respirator	Day 2	III, IV, VI
53	0.98	-0.63	-1.36	-0.83	0.76	40	M	Yes, respiratory	Day 14	VII
72	0.00	-1.11	-0.50	-0.52	35	M	Yes, respiratory, influenzavirus A	Day 4	No
4	-0.09	0.16	-0.05	-0.78	0.13	35	F	Yes, digestive	Day 3	No
10	-0.23	2.53	0.19	0.99	-0.05	38	M	Yes, respiratory	Day 7	III, IV, VI, IX, X
17	-0.06	0.56	-0.16	0.55	-0.83	68	F	Yes, respiratory	Day 10	III
18	-3.97	2.33	-0.38	-0.35	-0.03	23	F	Yes, respiratory	Day 4	III, IV, V, VI, VII, IX, X, X I
28	-0.30	0.14	-0.36	-1.34	-0.37	28	F	Yes, respiratory	Day 4	III, VI, VII, X I
31	-0.06	0.45	-0.40	-0.17	-0.57	25	M	Yes	Day 6	III, VI
45	-0.16	0.15	-1.08	-0.86	-0.04	58	F	Yes, respiratory	?	III, IV, VI
54	-0.75	0.71	-0.04	-1.24	0.23	50	M	Yes, respiratory, digestive	Day 7	No
77	0.32	0.58	-1.38	0.23	-1.07	56	F	Yes	Day 8	III, IV, VI, VII, IX, X, X I
93	-0.73	1.17	-0.65	-0.90	-1.33	42	F	Yes	Day 5	III, IV, VI, VII, IX, X, X I, X II
94	-0.24	1.23	0.17	0.26	0.29	55	M	Yes, upper airway	Day 5	III, IV, VI, IX, X, X II
1	0.98	-2.07	1.25	-0.62	-1.74	54	M	Yes	Day 5	No
2	-0.54	1.10	2.36	-1.00	0.92	60	F	Yes, digestive	Day 10	No
36	-0.49	1.30	0.06	-0.64	64	F	No	Day 9	No
55	-0.43	0.04	1.57	-0.52	-0.02	67	F	Yes, respiratory	?	III, IV, V, VI
56	-0.21	0.00	1.53	-1.79	-0.37	37	F	No	Day 7	No
64	0.01	0.09	0.79	-1.01	0.25	59	M	Yes, respiratory	Day 12	No
65	0.77	0.69	2.50	2.11	1.04	69	F	Yes, digestive	?	No
76	0.13	0.19	1.27	-0.45	0.00	60	M	Yes	Day 1	VI
86	-0.08	-0.06	0.52	-0.68	-0.11	52	M	Yes, digestive	Day 1	No
100	-0.42	-0.41	1.68	0.22	-0.60	70	F	Yes, digestive		No
23	0.17	0.29	-0.39	0.81	0.49	48	M	Yes, digestive, norovirus?	Day 14	VII, IX, X, X II
33	0.77	-0.11	0.17	2.10	-0.39	29	F	Yes, respiratory	Day 7	No
41	0.31	-0.61	-1.57	0.38	-0.48	30	M	No	Day 15	V, VI, X I
47	0.18	-0.09	-0.43	0.93	0.85	38	F	No	Day 7	IX, X, X II
73	-0.07	-0.21	-1.45	1.35	-0.92	63	F	Yes, respiratory, mycoplasma	Day 7	IX, X
74	0.16	-0.15	0.12	0.48	-0.42	36	F	Yes, digestive	Day 5	III
75	-0.34	0.06	0.60	1.51	-0.03	36	F	Yes, upper airway	Day 5	III
80	1.09	-1.00	0.42	1.34	0.81	38	M	Yes, respiratory	Day 5	No
83	0.32	0.24	-1.20	0.75	-1.48	44	M	Yes		VII, IX, X, X II
87	0.67	-0.14	-0.57	1.25	-0.35	41	M	Yes	Day 2	No
13	-4.26	-4.22	1.15	1.30	1.48	66	M	No	Day 1	VII, IX, X II
19	-0.36	-0.37	0.18	1.17	1.90	47	F	Yes, respiratory	Day 7	No
12	0.47	0.52	0.43	0.27	1.16	72	F	Yes, digestive	Day 7	No
48	-0.18	-0.20	0.19	-1.80	0.68	55	M	Yes, digestive	Day 5	VII, IX, X, X I, X II
52	0.20	0.27	0.29	-0.75	0.98	84	M	Yes	Day 5	VII, IX, X, X I, X II
59	0.96	0.88	-1.08	-0.12	3.11	58	M	Yes, respiratory	Day 10	No
85	1.13	-0.61	0.33	1.51	1.57	60	F	Yes, upper airway	Day 1	No
90	0.47	0.13	-0.33	-0.71	1.96	58	M	No	Day 5	No

Samples were sorted by highest score, shown in red, in 5 factors of each sample. In 'antecedent infection' column, "respiratory" and "digestive" indicate their apparatuses. In 'cranial nerve' column, the numbers (III, IV, V…) indicate a site of lesion, such as eye movement (III, IV and VI), trigeminal nerve (V), and facial nerve (VII).
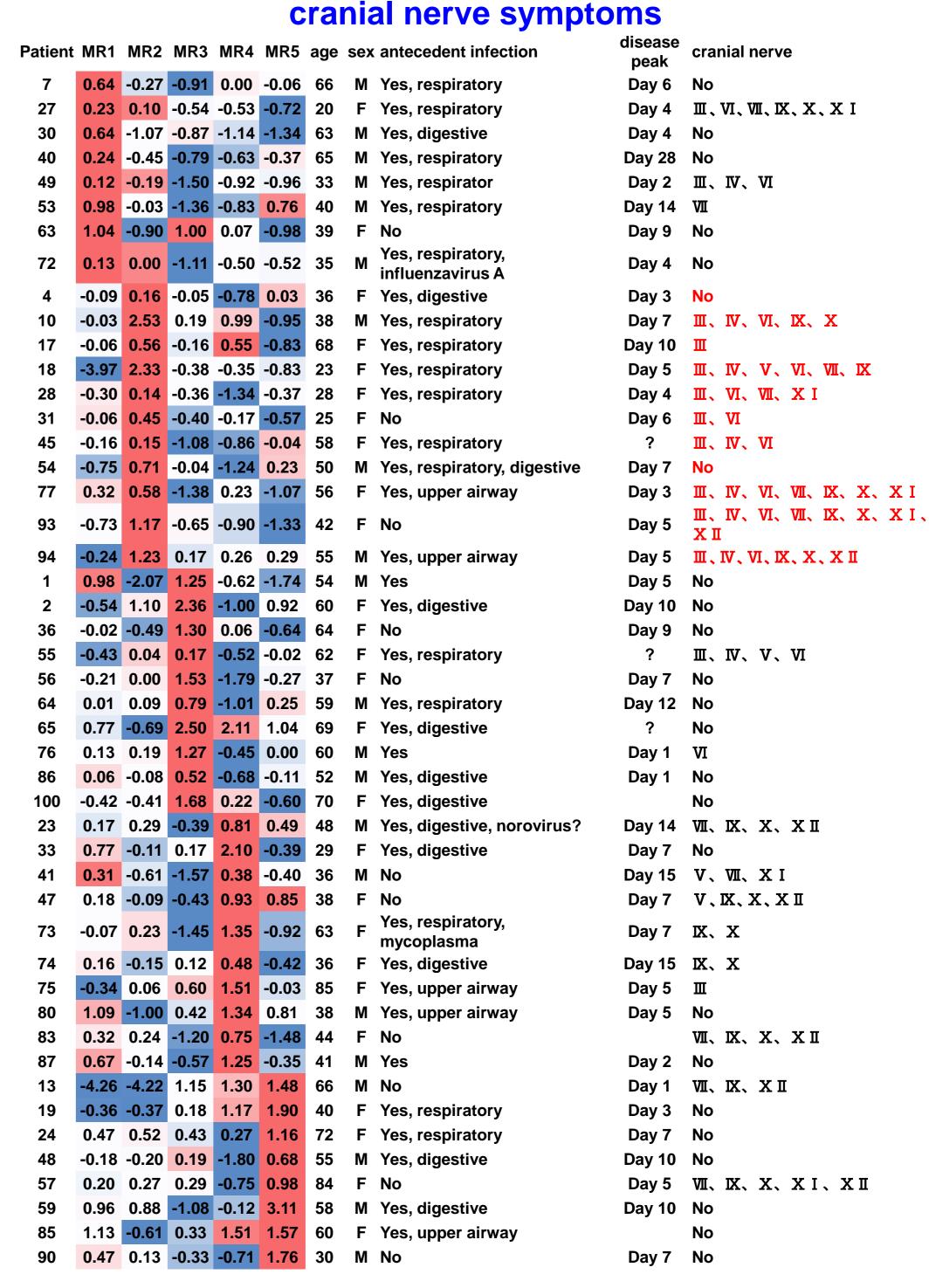
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Conclusions

- Hierarchical clustering and PCA don't work well for glycoarray data analysis.
- Factor analysis for glycoarray data extracts 5 factors, each of which was comprised of the distinct set of antibodies to glycolipids.
- Factor score associates M2, D1a, Q1b, and their complexes with cranial nerve symptoms.
- Future application of EFA of glycoarray data into more comprehensive data of GBS patients may give insight into the potential factors (or causes), which induce distinct antibodies against a set of glycolipids that are associated with specific clinical signs.