 doi:10.20944/preprints202212.0224.v1

Article

Autoantibody Correlation Signatures in Fibromyalgia and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Association with Symptom Severity

Varvara A. Ryabkova [1,2*], Natalia Y Gavrilova [1], Alina A Poletaeva [3], Alexander I Pukhalenko [3], Irina A Koshkina [4], Leonid P Churilov [1,5] and Yehuda Shoenfeld [1,6]

[1] Laboratory of the Mosaic of Autoimmunity and Department of Pathology, Saint Petersburg State University, Saint-Petersburg, Russia
[2] Pavlov First Saint Petersburg State Medical University, Saint-Petersburg, Russia
[3] Medical Research Centre "Immunculus", Moscow, Russia
[4] LLC "Doctor Koshkina", Moscow, Russia
[5] Saint Petersburg Research Institute of Phthisiopulmonology, 191036 Saint Petersburg, Russia.
[6] Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Ramat-Gan, 52621, Israel; Ariel University, Ariel, Israel
* Correspondence: varvara-ryabkova@yandex.ru;

Abstract: (1) Background: Recent studies provide some evidence for the contribution of antibody-mediated autoimmune mechanisms to the nature of fibromyalgia (FM) and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Much attention was paid to the autoantibodies (AAb) targeting G protein-coupled receptors as natural components of the immune system. However, natural AAb network is much more extensive, and has not been previously investigated in these disorders; (2) Methods: The enzyme immunoassays ELI-Viscero-Test and ELI-Neuro-Test were used to determine changes in serum content of a 33 natural AAb to neural, organ-specific and non-tissue-specific autoantigens a) in 11 FM patients with comorbid ME/CFS; b) in 11 ME/CFS patients without FM; c) in 11 healthy controls. Individual autoantibody profiles and their correlation with some clinical symptoms were analyzed. (3) Results: both patients with ME/CFS and ME/CFS+FM were characterized by more frequent and pronounced deviations in the immunoreactivity to GABA-receptors than healthy controls. Although the level of other natural AAb did not differ between study groups, AAb correlation signatures were changing in patients compared to healthy controls. Both in patients and healthy controls the level of natural AAb to various neural and tissue-specific antigens correlated with the severity of fatigue, bodily pain, depression, anxiety, physical and mental-health related quality of life. Notably, that widely different correlation patterns were observed between study groups. (4) Conclusions: Findings from this pilot study provide some evidence that the homeostasis of autoimmune relationships, which are possibly a physiological part of our immune system, may break down in FM and ME/CFS. The correlation of disease-induced perturbations in individual AAb profiles with some clinical symptoms may arise from the immune system's ability to reflect qualitative and quantitative changes in antigenic composition of the body.

Keywords: fibromyalgia; myalgic encephalomyelitis/chronic fatigue syndrome; autoantibodies; autoimmunity

1. Introduction

Fibromyalgia (FM) is a common cause of chronic, widespread, musculoskeletal pain with unclear etiopathogenesis. However, it was demonstrated in a recent breakthrough study that IgG from the patients of FM produced in mice painful sensory hypersensitivities by sensitizing peripheral nociceptive afferents[1]. IgG from patients in this study labeled satellite glial cells and neurons in vivo and in vitro, as well as human dorsal root ganglia as well as myelinated fiber tracts and a small number of macrophages and endothelial cells in mouse dorsal root ganglia. The same author group further showed that a subset of FM patients had elevated levels of anti-spinal ganglia autoantibodies (AAb) (detected in a cell culture assay), which were associated with more severe symptoms[2]. However, a large variation between individual serum samples was observed, suggesting that only a subset of FM patients have autoreactive IgG to spinal ganglia.

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a disabling clinical condition characterized by unexplained and persistent post exertional fatigue accompanied by a variety of symptoms related to cognitive, immunological, endocrinological, and autonomous dysfunction[3]. The common core symptoms of fatigue, sleep problems and cognitive difficulties lead to significant comorbidity between FM and ME/CFS, but it can vary depending on the diagnostic criteria used[4]. While in one study 34% of 313 patients diagnosed with ME/CFS had comorbid FM according to the old American College of Rheumatology (ACR) old diagnostic criteria of 1990, the other research group reported that the prevalence of FM among patients with ME/CFS could reach 50% when ACR 2010 newer diagnostic criteria were applied[4]. The recent meta-analysis confirmed prominent clinical overlap between FM and ME/CFS[5]. Although the exact pathogenesis of ME/CFS is still unknown, according to the most widely held hypothesis, it is a complex multifactorial syndrome with immunological, metabolic, mitochondrial, autonomic and adrenal dysfunction as the key pathogenetic mechanisms[6,7]. Emerging evidence suggests that disorders of autoimmunity play an important role in postinfectious ME/CFS and that targeting autoantibodies could be a promising treatment approach[8].

At the same time, increased level of any AAb in the serum of patients is not an ultimate sign of autoimmune disease, but can indicate changes in expression and/or excretion of the corresponding antigens[8]. From this point of view, not only increased, but also a decreased levels of AAb are relevant. Recently, a network of natural AAb against adrenergic, muscarinic and other G-protein coupled receptors (GPCR) has been described which was shown to be dysregulated in a wide spectrum of diseases (not only autoimmune ones)[9]. We assume that AAb to GPCR are only a special case of a complex natural AAb network. The purpose of our study was to compare these networks between patients suffered from ME/CFS with and without comorbid FM and health controls.

2. Materials and Methods

2.1. Patients and controls

Patients were included in the study if they met all three the most commonly used sets of ME/CFS diagnostic criteria (Fukuda et al. (1994) CFS criteria[10], the Canadian Consensus criteria of ME/CFS (2003) [11], and US Institute of Medicine, now called the National Academy of Medicine (IOM/NAM) criteria (2015)[12]). Patients were assigned to the ME/CFS(+)FM or ME/CFS(-)FM groups depending on whether they met ACR 2016 diagnostic criteria for FM[13]. The third group consisted of apparently healthy controls (HC). Individuals with any autoimmune disease and those who had any acute illness during last 3 months were excluded from the study. The study was approved by the Ethics Committee of Saint Petersburg State University. All participants gave informed consent.

2.3. Questionnaires for Symptom Scoring

Both patients and HC were assessed for the clinical symptoms (including depression and anxiety) and baseline health status using the following instruments: the Short Form 36 Health Survey (SF-36), the Multidimensional Fatigue Inventory (MFI), DePaul Symptom Questionnaire-Short form (DSQ-SF), and Hospital Anxiety and Depression Scale (HADS). The SF-36 includes the following subject-reported evaluations about current health status: physical and social functioning, physical and emotional limitations, vitality, pain, and general and mental health[14]. The MFI comprises of a 20-item self-reported

 doi:10.20944/preprints202212.0224.v1

questionnaire focused on general, physical and mental fatigue, reduced activity, and reduced motivation[15]. Cognitive symptoms were tested based on the self-reported DSQ-SF questionnaire data. In particular, composite scores were calculated for cognitive symptoms by averaging the scores for the frequency and severity (ranged from 0 to 4) of two symptoms of cognitive dysfunction, included in DSQ-SF questionnaire (“Problems remembering things” and “Difficulty paying attention for a long period of time”). HADS is a reliable scale for identifying and assessing the severity of symptoms of anxiety disorders and depression, both among patients with somatic diseases and among patients with mental disorders[16]. The score 0–7 in each subscale (depression and anxiety symptoms) represents “normal,”; 8-10 points - "borderline results or doubtful case of anxiety / depression"; 11 points or more - "probable case of anxiety / depression"

2.2. Autoantibody quantification - ELISA

We defined individual normalized levels of AAb against 21 organ-specific and non-organ-specific antigens and 12 neural antigens using standardized ELISA test systems for semi-quantitave serum AAb evaluation (ELI-Viscero-test-24 and ELI-N-test-12 by Medical Research Center “Immunculus”, Moscow, Russia). The antigens used in the test systems are listed in Table 1.

Table 1. List of antigens, included in the test systems ELI-Viscero-test-24 and ELI-N-test-12

№	Antigen	Abbreviation
1	Double stranded deoxyribonucleic acid	dsDNA
2	β2-glycoprotein-I	β2-GP
3	Fc-fragments of IgG	Fc-IgG
4	Collagen type IV	Collagen
5	Membrane antigen of cardiomyocytes	CoM
6	β1-adrenergic receptors of cardiomyocytes	β1 Adr Re
7	Platelet membrane antigen	TrM-03
8	Cytoplasmic antigen of neutrophils	ANCA
9	Membrane antigen of renal glomerular cells	KiM-05
10	Membrane antigen of pulmonary alveolocytes	LuM-02
11	Membrane antigen of gastric wall cells	GaM-02
12	Membrane antigen of small intestine wall cells	ItM-07
13	Membrane antigen of colon wall cells	ScM
14	Cytoplasmic antigen of hepatocytes	HeS-08
15	Membrane antigen of hepatocyte mitochondria	HMMP
16	Human insulin	Insulin
17	Insulin receptors	Ins-Re
18	Thyroglobulin	TG
19	Thyrotropin receptor	TSH-Re
20	Membrane antigen of adrenal medulla cells	Adr-D\C
21	Membrane antigen of sperm and prostate cells	SPr-06
22	Neurofilament protein 200	NF-200
23	Glial fibrillary acidic protein	GFAP
24	S100 protein	S100
25	Myelin basic protein	MBP
26	Voltage-dependent calcium channel	V-Ca-Channel
27	N-cholinergic receptors	Ach-Re
28	Glutamate receptors	Glu-Re
29	γ-aminobutyric acid receptors	GABA-Re

 doi:10.20944/preprints202212.0224.v1

30	Dopamine receptors	Dopa-Re
31	Serotonin receptors	5HT-Re
32	μ-opioid receptors	μ-Opioid-Re
33	β-endorphin	β-Endorphin

The pooled control serum was a preparation of polyclonal immunoglobulins of the IgG class, synthesized by B-lymphocytes in response to antigenic stimuli that occurred throughout the life of donors. Immunoglobulins in the control serum were obtained from the blood serum of more than 5000 healthy donors and brought to a concentration close to physiological (16 mg/mL). Thus, pooled control serum contained population-normalized IgG class polyclonal antibodies to each of the studied antigens and was used as a universal standard for all tested antigens. Depending on the studied antigen, the pooled control serum was diluted to a final concentration, which was derived on the basis of studies of the level of autoantibodies of a large cohort of individual serum samples from healthy donors. The content of AAb to the studied antigens was evaluated in the conventional units of optical density and compared to their content in a control pool of sera from healthy donors (taken for 100%). Then the average individual immunoreactivity (AIR) of the studied samples for each of the antigens was calculated in comparison with pooled control serum according to the formula:

$$\mathrm{AIR} = \frac{\frac{R(ag1)*100}{R(k1)} - 100 + \frac{R(ag2)*100}{R(k2)} - 100 + \cdots + \frac{R(agN)*100}{R(kN)} - 100}{24}$$

AIR – the average reactivity of an individual patient's serum to all studied antigens, expressed as a percentage of the average reactivity of the pooled control serum with the same antigens.

R (ag1, 2,…N) – reactivity (in units of optical density) of the patient's serum with studied antigens

R(k1, k2, …N) – reactivity (in units of optical density) of pooled control serum with studied antigens

The normal (physiological) levels of individual AIR are restricted by the ranges −30% ··· to 0% (or conditional units (CU)) of the control sample AIR.

To construct immunoreactivity profiles, the deviation (as a percentage of the individual AIR) of the patient's serum reactivity with each of the antigens was calculated using specialized software according to the formula:

$$R(dev)agN = \left(\frac{R(agN)*100}{R(kN)}\right) - 100 - AIR$$

R(dev)agN – deviation (as a percentage of the AIR) of the patient's serum reactivity with antigen N;

R (agN) – reactivity (in units of optical density) of the patient's serum with studied antigens

R(kN) – reactivity (in units of optical density) of pooled control serum with studied antigens

The normal (physiological) R(dev)agN for each AAb is restricted by the range from −15% ··· to +10% (or conditional units (CU)) from the individual AIR.

2.3. Statistical Analysis

Statistical processing was performed with the Statistica 10.0 software package. To compare the prevalence of non-physiological AAb deviations from the individual AIR Chi-squared test was applied. To compare mean R(dev)agN values between patients and HC Mann–Whitney U test was applied. In order to compare AAb correlation signatures between groups, Spearman correlation analysis was performed in each group. We used Chord diagrams to visualize the patterns of Spearman's rank correlation coefficients between AAb. Spearman correlation analysis was also performed to study relationship between the severity of different symptoms and R(dev)agN values. Differences were considered significant at $p < 0.05$.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2022 doi:10.20944/preprints202212.0224.v1

3. Results

3.1. Subject Characteristics

The study involved 11 patients with ME/CFS+FM, 11 patients with ME/CFS who did not suffer from FM and 11 healthy controls. Patient characteristics are shown in Table 2.

Table 2. Study population characteristics. BMI – body mass index, FM – fibromyalgia, HC – heathy controls, IQR – interquartile range, ME/CFS - myalgic encephalomyelitis/chronic fatigue syndrome.

Subject Characteristics		ME/CFS(+)FM (n=11)	ME/CFS(-)FM (n=11)	HC (n=11)	p-values[1] ME/CFS(+)FM vs HC	p-values[1] ME/CFS(-)FM vs HC
Gender	Female	8	9	8	1.000	0.655
	Male	3	2	3		
Age	Median (IQR)	38.0 (31.0-47.0)	30.0 (27.0-45.0	33.0 (27.0-49.0) 1	0.519	0.562
BMI	Median (IQR)	18.8 (17.9-19.6)	23.8 (19.8-32.3)	21.4 (19.3-25.4)	0.010	0.193
Illness Duration (years)	Median (IQR)	6.0 (3.0-21.5)	7.0 (6.0-10.5)	N/A	N/A	
Onset of disease	Infection-triggered	4	8	N/A	N/A	
	Severe stress(-es)-triggered	5	2			

1 – For categorial variable p-values were derived from Chi-squared test; for continuous variables, p-values were derived from Mann–Whitney U test.

ME/CFS(+)FM and ME/CFS(-)FM did not differ from controls in terms of gender and age. While BMI in ME/CFS(-)FM group was similar to the BMI in HC, it was significantly lower in ME/CFS(+)FM group. Regarding race and ethnicity, all patients and HC were Caucasians and not Hispanic.

The illness duration varied, with a range of 1 to 35 years and median value of 6.0 years in ME/CFS(+)FM group and with a range of 2,5 to 35 years and median value of 7.0 years in ME/CFS(+)FM group . 4 patients (33%) with ME/CFS and comorbid FM and 8 patients (73%) without comorbid FM reported an infection-triggered onset of disease.

All scales from SF-36 and MFI were significantly different between ME/CFS(+)FM and heathy controls as well as between ME/CFS(-)FM and healthy controls (Table 3). Both physical and mental component scores (PCS and MCS, respectively) derived from the SF-36 short survey, were, as expected, higher in the control group ($p < 0.05$), indicating better health.

Depression and anxiety levels were significantly higher in the groups of ME/CFS(-)FM and ME/CFS(+)FM. The median HADS-D subclase score indicated probable comorbid depression only in ME\CFS(+)FM group, and the median HADS-A score corresponded to borderline anxiety in both groups of patients.

Table 3. Clinical symptoms, depression, anxiety, and baseline health status assessment. FM – fibromyalgia, HADS - Hospital Anxiety and Depression Scale, HC – heathy controls, IQR – interquartile range, PCS - physical component score; MCS - mental component score, ME/CFS - myalgic encephalomyelitis/chronic fatigue syndrome, MFI - multidimensional Fatigue Inventory.

Subject Characteristics	ME/CFS(+)FM (n=11)	ME/CFS(-)FM (n=11)	HC (n=11)	p-values ME/CFS(+) FM vs HC	p-values ME/CFS(-) FM vs HC

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2022 doi:10.20944/preprints202212.0224.v1

SF-36 Scales Median (IQR)	Physical Functioning	40.0 (30.0-55.0)	45.0 (30.0-75.0)	100.0 (95.0-100.0)	0.000	0.000
	Role physical	0.0 (0.0-0.0)	0.0 (0.0-0.0)	100.0 (50.0-100.0)	0.000	0.000
	Bodily pain	41.0 (31.0-41.0)	74.0 (22.0-100.0)	100.0 (84.0-100.0)	0.000	0.028
	General Health	35.0 (20.0-45.0)	30.0 (25.0-40.0)	87.0 (62.0-92.0)	0.000	0.000
	Vitality	10.0 (0.0-25.0)	10.0 (0.0-20.0)	80.0 (55.0-85.0)	0.000	0.000
	Social functioning	25.0 (0.0-50.0)	25.0 (0.0-37.5)	100.0 (75.0-100.0)	0.000	0.000
	Role emotional	33.3 (0.0-100.0)	0.0 (0.0-100.0)	100.0 (66.7-100.0)	0.040	0.028
	Mental health	52.0 (28.0-56.0)	40.0 (28.0-56.0)	68.0 (52.0-80.0)	0.002	0.001
	PCS	30.6 (26.3-35.0)	35.1 (30.0-45.5)	57.2. (50.8-58.7)	0.000	0.000
	MCS	34.5 (21.1-43.3)	26.0 (21.9-37.0)	53.1 (44.5-55.1)	0.001	0.000
MFI Scales Median (IQR)	General Fatigue	19.0 (18.0-20.0)	19.0 (19.0-20.0)	7.0 (6.0-9.0) 1	0.000	0.000
	Mental Fatigue	16.0 (13.0-18.0)	14.0 (9.0-15.0)	6.0 (5.0-10.0)	0.000	0.010
	Physical Fatigue	16.0 (15.0-20.0)	18.0 (16.0-20.0)	6.0 (5.0-9.0)	0.000	0.000
	Reduced Activity	19.0 (16.0-20.0)	19.0 (17.0-20.0)	9.0 (5.0-11.0)	0.000	0.000
	Reduced Motivation	14.0 (9.0-17.0)	13.0 (11.0-14.0)	9.0 (5.0-11.0)	0.003	0.001
HADS Median (IQR)	Depression subscale	13.0 (10.0-15.0)	11.0 (9.0-16.0)	3.0 (0.0-4.0)	0.000	0.000
	Anxiety subscale	10.0 (7.0-14.0)	10.0 (6.0-12.0)	4.0 (1.0-5.0)	0.001	0.003

A significantly higher proportion of patients in ME/CFS(-)FM and ME/CFS(+)FM groups presented with abnormal levels of AAbs against GABA receptors than healthy controls (Table 4). At the same time, the proportion of patients with abnormal peaks of any other AAb in groups of patients did not differ significantly from the controls.

Table 4 Number of patients and HC with abnormal deviation (as a percentage of the AIR) of the patient's serum reactivity towards the studied antigens. FM – fibromyalgia, HC – heathy controls, ME/CFS - myalgic encephalomyelitis/chronic fatigue syndrome.

Autoantibodies	ME/CFS(+)FM (n=11)		ME/CFS(-)FM (n=11)		HC (n=11)		p-values ME/CFS(+)FM vs HC	p-values ME/CFS(-) FM vs HC
Anti-dsDNA AAb	2	(18,2%)	3	(27,3%)	2	(18,2%)	1.00	0.66
Anti- β2-GP AAb	0	(0,0%)	3	(27,3%)	1	(9,1%)	1.00	0.57
Anti-Fc-IgG AAb	1	(9,1%)	1	(9,1%)	2	(18,2%)	0.61	0.61
Anti Collagen AAb	4	(36,4%)	1	(9,1%)	3	(27,3%)	0.68	0.34
Anti-CoM AAb	1	(9,1%)	2	(18,2%)	2	(18,2%)	0.61	1.00
Anti β1 Adr Re AAb	2	(18,2%)	2	(18,2%)	3	(27,3%)	1.00	0.66
Anti TrM-03 AAb	1	(9,1%)	0	(0,0%)	3	(27,3%)	0.34	0.11
Anti ANCA AAb	1	(9,1%)	2	(18,2%)	2	(18,2%)	0.61	1.00
Anti KiM-05 AAb	1	(9,1%)	1	(9,1%)	1	(9,1%)	1.00	1.00

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2022 doi:10.20944/preprints202212.0224.v1

Anti LuM-02 AAb	4	(36,4%)	5	(45,5%)	6	(54,5%)	0.43	0.70
Anti GaM-02 AAb	1	(9,1%)	3	(27,3%)	2	(18,2%)	0.61	0.66
Anti ItM-07 AAb	5	(45,5%)	3	(27,3%)	5	(45,5%)	1.00	0.42
Anti ScM AAb	4	(36,4%)	3	(27,3%)	1	(9,1%)	0.17	0.34
Anti HeS-08 AAb	0	(0,0%)	4	(36,4%	1	(9,1%)	1.00	0.53
Anti HMMP AAb	1	(9,1%)	0	(0,0%)	2	(18,2%)	1.00	0.24
Anti Insulin AAb	1	(9,1%)	2	(18,2%)	1	(9,1%)	1.00	0.61
Anti Ins-Re AAb	1	(9,1%)	0	(0,0%)	1	(9,1%)	1.00	1.00
Anti TG AAb	0	(0,0%)	3	(27,3%)	0	(0,0%)	1.00	0.11
Anti TSH-Re AAb	1	(9,1%)	1	(9,1%)	1	(9,1%)	1.00	1.11
Anti-Adr-D\C AAb	1	(9,1%)	3	(27,3%)	2	(18,2%)	0.61	0.66
Anti-SPr-06 AAb	2	(18,2%)	4	(36,4%)	3	(27,3%)	0.66	0.68
Anti-NF-200 AAb	0	(0,0%)	1	(9,1%)	1	(9,1%)	1.00	1.00
Anti-GFAP AAb	1	(9,1%)	0	(0,0%)	0	(0,0%)	1.00	1.00
Anti-S100 AAb	1	(9,1%)	1	(9,1%)	1	(9,1%)	1.00	1.00
Anti-MBP AAb	0	(0,0%)	0	(0,0%)	2	(18,2%)	1.00	0.48
Anti-V-Ca-Channel AAb	0	(0,0%)	1	(9,1%)	1	(9,1%)	1.00	1.00
Anti-Ach-Re AAb	0	(0,0%)	1	(9,1%)	0	(0,0%)	1.00	1.00
Anti-Glu-Re AAb	1	(9,1%)	0	(0,0%)	2	(18,2%)	1.00	0.48
Anti-GABA-Re AAb	6	(54,5%)	5	(45,5%)	0	(0,0%)	0.0	0.06
Anti-Dopa-Re AAb	2	(18,2%)	2	(18,2%)	2	(18,2%)	1.00	1.00
Anti-5HT-Re AAb	1	(9,1%)	1	(9,1%)	1	(9,1%)	1.00	1.00
Anti- μ-Opioid-Re AAb	1	(9,1%)	2	(18,2%)	1	(9,1%)	1.00	0.61
Anti- β -Endorphin AAb	1	(9,1%)	3	(27,3%)	2	(18,2%)	0.61	0.66

In order to assess the extent of abnormalities in the immunoreactivity profiles of patients, we calculate median absolute deviations of the participants' serum reactivity towards each of the analyzed antigens from AIR (median R(dev)agNs) in each of the three study groups, and then compare the obtained values between patients and controls. No significant differences have been observed.

Based on the concept of antibodiome as a functional and physiological network of AAbs, which reflects the exposome and could be disturbed in the disease process[17], we performed a correlation analysis between R(dev)agNs and some clinical characteristics (BMI, age, SF-36 and MFI-20 subscales, depression, anxiety, composite score for cognitive symptoms frequency and severity) in each of the three study groups. The significant correlations are shown in Tables 5,6,7.

Table 5 Clinical correlations of the absolute AAb deviations from AIR in the group of patients suffered from ME/CFS without comorbid FM. (Spearman correlation coefficient r and p-value)

Subject Characteristics		Autoantibodies	r	p
SF-36 Scales Median (IQR)	Bodily pain	Anti KiM-05 AAb	+0,611	0,046
		Anti ScM AAb	+0,752	0,008
		Anti HMMP AAb	+0,795	0,003
	Physical component score	Anti ItM-07 AAb	+0,724	0,012
	Mental component score	No significant correlation was found		

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2022 doi:10.20944/preprints202212.0224.v1

MFI Scales Median (IQR)	General Fatigue	Anti ItM-07 AAb	-0,659	0,027
		Anti-SPr-06 AAb	+0,697	0,017
	Mental Fatigue	No significant correlation was found		
	Physical Fatigue	No significant correlation was found		
	Reduced Activity	Anti-V-Ca-Channel AAb	-0,620	0,042
		Anti-Ach-Re AAb	+0,623	0,041
	Reduced Motivation	Anti β Endorphin AAb	+0,665	0,026
HADS	Depression subscale	Anti-GABA-Re AAb	-0,639	0,034
		Anti-SPr-06 AAb	-0,639	0,034
	Anxiety subscale	Anti-Dopa-Re AAb	-0,762	0,006
		Anti-MBP AAb	0,820	0,002
DSQ-SF	Composite score of cognitive symptoms	No significant correlation was found		
	Illness duration	Anti-Dopa-Re AAb	-0,652	0,03
		Anti-GFAP AAb	+0,668	0,025
	Age	Anti TG AAb	+0,728	0,011
		Anti-Fc-IgG AAb	-0,741	0,009
		Anti GaM-02 AAb	-0,636	0,035
		Anti HeS-08 AAb	-0,800	0,003
	BMI	No significant correlation was found		

Table 6 Clinical correlations of the absolute AAb deviations from AIR in the group of patients suffered from ME/CFS with comorbid FM. (Spearman correlation coefficient r and p-value)

Subject Characteristics		Autoantibodies	r	p
SF-36 Scales Median (IQR)	Bodily pain	Anti-CoM AAb	+0,685	0,04
	Physical component score	No significant correlation was found		
	Mental component score	Anti-GFAP AAb	-0,774	0,005
		Anti ItM-07 AAb	+0,612	0,046
	General fatigue	Anti-V-Ca-Channel AAb	+0,675	0,023

doi:10.20944/preprints202212.0224.v1

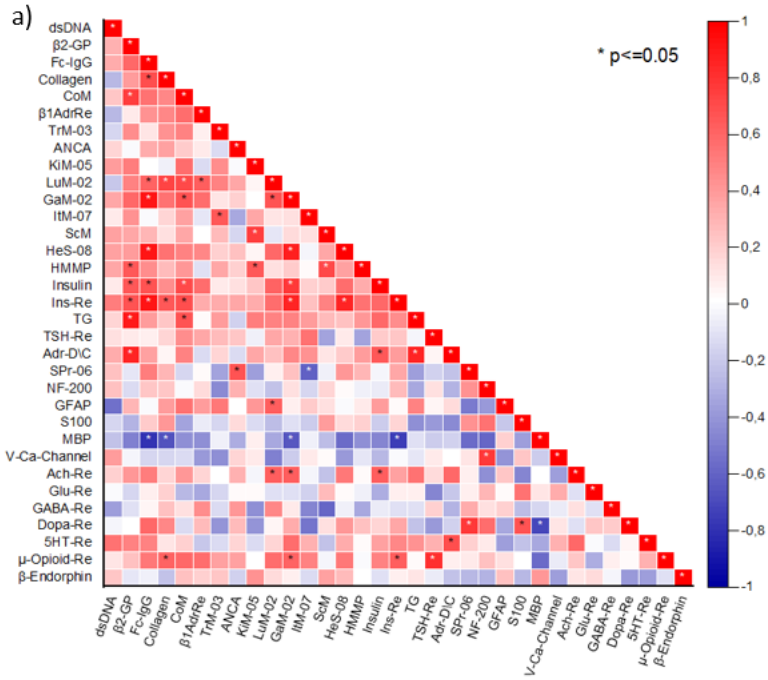
	Mental Fatigue	Anti β Endorphin AAb	+0,773	0,005
	Physical Fatigue	Anti-V-Ca-Channel AAb	+0,646	0,032
		Anti TG AAb	-0,785	0,004
	Reduced Activity	Anti TG AAb	-0,613	0,045
		Anti- SPr-06 AAb	-0,832	0,001
		Anti β Endorphin AAb	+0,629	0,038
	Reduced Motivation	Anti-5HT-Re AAb	-0,772	0,005
HADS	Depression subscale	Anti- β2-GP AAb	-0,629	0,038
		Anti ItM-07 AAb	-0,627	0,039
	Anxiety subscale	Anti-dsDNA AAb	+0,700	0,017
DSQ-SF	Composite score of cognitive symptoms	Anti-MBP AAb	+0,617	0,043
	Illness duration	No significant correlation was found		
Age		No significant correlation was found		
BMI		Anti-dsDNA AAb	+0,662	0,026

Table 7 Clinical correlations of the absolute AAb deviations from AIR in HC group. (Spearman correlation coefficient r and p-value)

Subject Characteristics		Autoantibodies	r	p
SF-36 Scales Median (IQR)	Bodily pain	Anti-CoM AAb	+0,774	0,005
		Anti- μ-Opioid-Re AAb	-0,719	0,013
	Physical component score	Anti-CoM AAb	+0,636	0,035
	Mental component score	Anti-Dopa-Re AAb	-0,637	0,035
		Anti-GABA-Re AAb	-0,664	0,026
MFI Scales Median (IQR)	General Fatigue	No significant correlation was found		
	Mental fatigue	Anti-CoM AAb	-0,647	0,031
	Physical Fatigue	No significant correlation was found		
	Reduced Activity	Anti-Adr-D\C AAb	-0,644	0,033
	Reduced Motivation	No significant correlation was found		
HADS	Depression subscale	Anti LuM-02 AAb	-0,617	0,043
		Anti-Adr-D\C AAb	-0,683	0,020
	Anxiety subscale	Anti- μ-Opioid-Re AAb	+0,817	0,002
		Anti-Ach-Re AAb	+0,696	0,017
		Anti-Dopa-Re AAb	+0,646	0,032

DSQ-SF	Composite score of cognitive symptoms	Anti- μ-Opioid-Re AAb	+0,861	0,001
Age		Anti Ins-Re AAb	-0,657	0,028
BMI		Anti-CoM AAb	-0,737	0,010
		Anti ItM-07 AAb	-0,618	0,043
		Anti HeS-08 AAb	-0,706	0,015
		Anti TG AAb	-0,679	0,022
		Anti-S100 AAb	+0,632	0,037

To identify changes in the AAb relationships in patients suffered from ME/CFS with and without concominant FM, we analyzed AAb correlations in the two patients groups separately. A number of correlations between the absolute R(dev)agNs values in both groups were revealed. Disease-specific changes among the studied AAb were identified (Figure 1a, 1b).



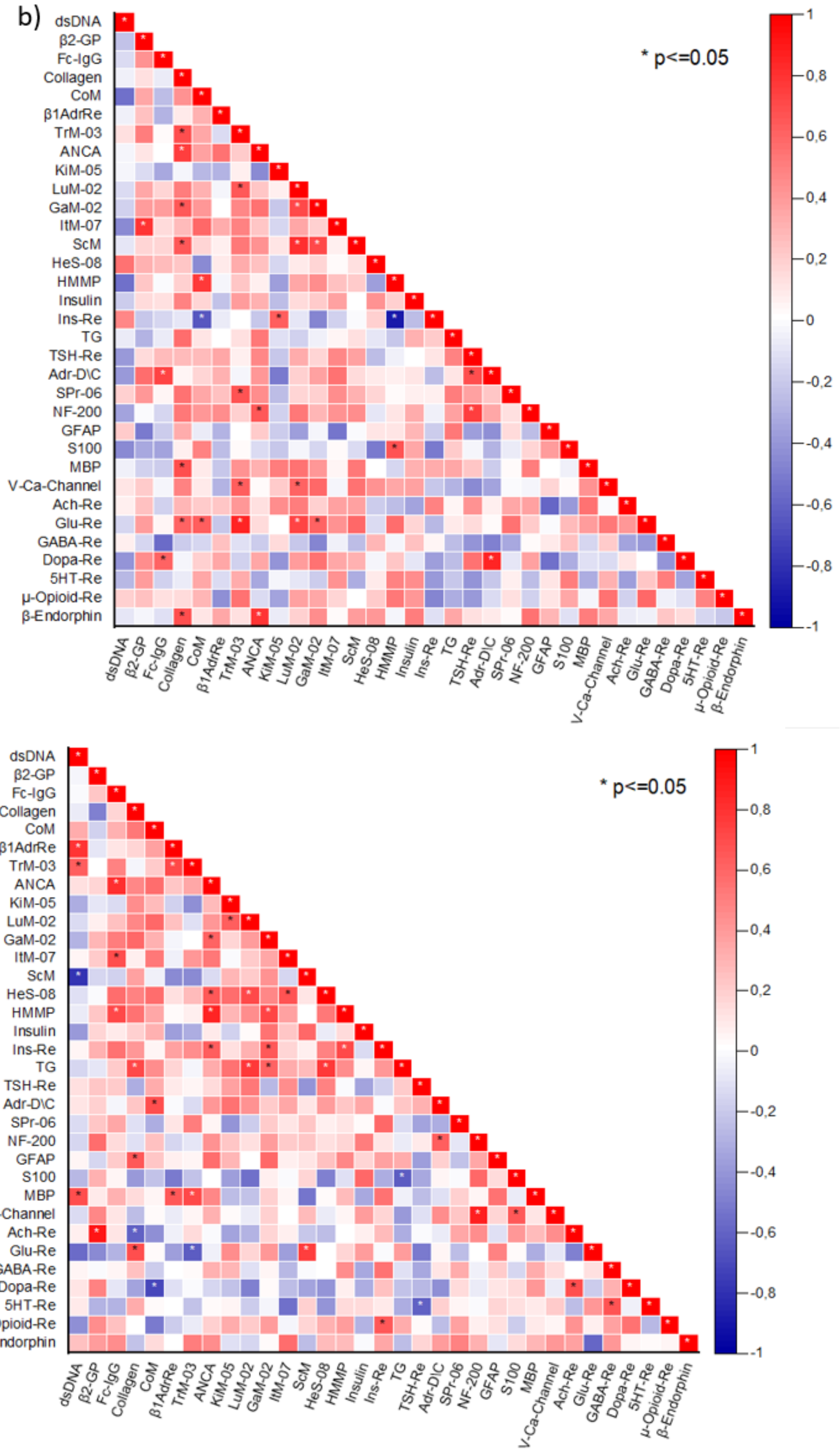
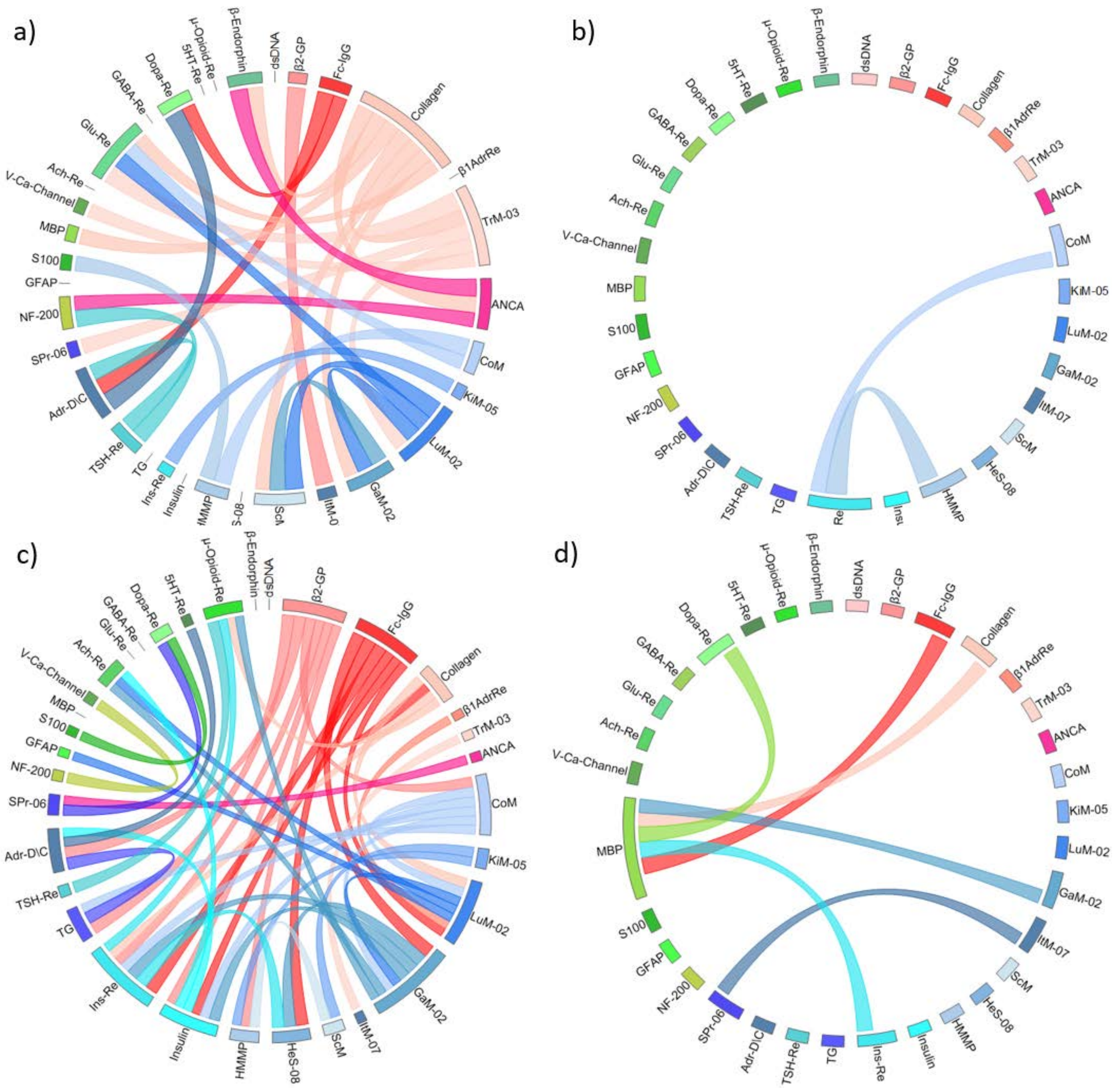


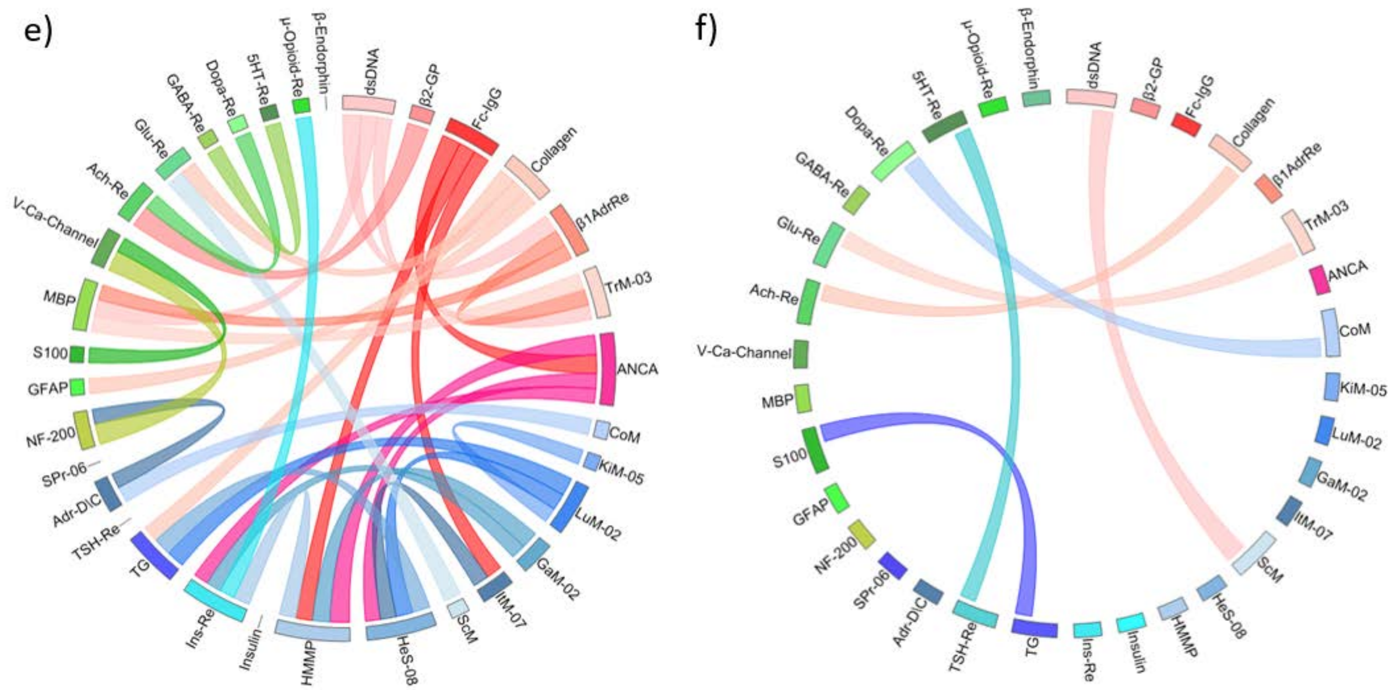
Figure 1 Correlation matrices of AAb targeting organ-specific, neural and non-organ-specific antigens (denoted by abbreviations as per legend in Table 1) for a) ME/CFS(-)FM (n = 11), b) ME/CFS(+)FM (n = 11), c) healthy controls (n = 11). The color scale bar represents the range of Spearman's rank correlation coefficient. Significant correlations are marked with *

The aforementioned data on the correlation between R(dev)agNs and some symptoms score even in HC suggested the presence of physiological relationships among natural AAb. Based on these findings, we expanded our study to include the analysis of HC sera for correlations between the studied AAb (Figure 1c). As previously shown for a few types of AAb (especially those targeting GPCR)[9], we observed that a number of AAb targeting various organ-specific, neural and non-organ-specific antigens correlated with each other in HC.

To gain better insights into the antibodiom, we presented the significant AAb correlations for each study cohort in chord diagrams. The relationship between the AAb from three antigen groups (organ-specific, neural and non-organ-specific), the number of AAb correlations in patients and HC, and the loss of normal correlation signatures in the disease are more descriptive in these plots (Figure 2 a-f)


a)
b)
c)
d)
β-Endorphin
μ-Opioid-Re
5HT-Re
Dopa-Re
GABA-Re
Glu-Re
Ach-Re
V-Ca-Channel
MBP
S100
GFAP
NF-200
SPr-06
Adr-D\C
TSH-Re
TG
Ins-Re
Insulin
HMMP
HeS-08
ScM
ItM-07
GaM-02
LuM-02
KiM-05
CoM
ANCA
TrM-03
β1AdrRe
Collagen
Fc-IgG
β2-GP
dsDNA

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2022 doi:10.20944/preprints202212.0224.v1



.Figure 2 Chord diagrams show the correlation matrix of AAb comparing the study cohorts. Segments in circles indicate studied AAb (see Table 1 for abbreviations employed), which are grouped according to the target antigen (red/pink = organ-specific, green = neural, blue = non-organ-specific antigens). Chords linking AAb indicate significant correlations (at least $p < 0.05$) according to Spearman rank correlations, while chord thickness is directly proportional to correlation coefficient. For clarity, positive and negative correlations are shown separately. A) Positive correlations, ME/CFS(+)FM; b) Negative correlations, ME/CFS(+)FM; c) Positive correlations, ME/CFS(-)FM; d) Negative correlations, ME/CFS(-)FM; e) Positive correlations, HC; f) Negative correlations, HC.

4. Discussion

The role of autoimmunity in ME/CFS and FM is widely discussed now, largely due to the emerging data on functional anti-GPCR AAB in ME/CFS[8] and successful passive transfer of FM pain from patients to mice[1]. At the same time, it should be kept in mind that the presence of AAb does not imply of the presence of autoimmue condition, as AAb are also recognized in non-autoimmune diseases[18,19]. According to the modern interpretation of the phenomenon of physiological autoimmunity, AAb act as adaptive bioregulators of cell functions and growth such as neurotransmitters or hormones both in health and disease[20]. Moreover, there is a concept of "Immunculus" or "Immune Homuculus" based on the assumption that the network of physiological autoreactive antiidiotypic autoantibodies may dynamically reflect the whole individual antigenome as a totality of internal im-munological images of the autoantigens[21–23].

The results, obtained in our study suggest that ME/CFS and FM are rather not autoimmune diseases, but the conditions with dysregulated natural autoimmunity. In particular, while 54,5% and 45,5% of patients in ME/CFS(+)FM and ME/CFS(-)FM groups presented with abnormal absolute deviations of the patients' serum reactivity against GABA Re compared to none in the control group, but these changes were not very pronounced (since no significant differences have been observed in median R(dev)agNs between the three groups). Classical autoimmune diseases with pathogenic AAb are characterized with a significant increase in the level of the AAb.

According to the concept of physiological autoaimmunity, quantitative changes in the content of natural AAb are related to variations of expression and secretion of the relative antigen, reflecting functional state of the corresponding cell type[17,23]. GABA Re is an element of endogenous stress-regulating mechanisms, preventing distress[24]. The last factor has long been considered increasing a risk of ME/CFS[25]. An imbalance

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 December 2022 doi:10.20944/preprints202212.0224.v1

between excitatory and inhibitory neurotransmission has been linked to ME/CFS and FM[26]. Interestingly, that pregabalin, which is one of the three FDA-approved drugs for the treatment of FM, is a lipophilic analogue of GABA. At the same time, it neither acts like GABA nor binds to GABA receptors, but binds strongly to the auxiliary alpha-2 delta subunit of the presynaptic voltage-gated calcium channel receptor to reduce the activation of postsynaptic neurotransmitter release[26]. Regarding treatment implications of our findings, it should be mentioned that a number of complementary dietary supplements have been reported to rebalance of glutamate:GABA, namely Omega-3 PUFAs, CoQ10, Withania Somnifera (Ashwagandha, Indian Ginseng), N-acetylcysteine, vitamin B12, curcumin (contained in turmeric), zinc, magnesium, 2-aminoethanesulfonic acid (L-Taurine), and carnitine (L-Carnitine)[27].

The majority of Aab, for which significant correlations with the symptom scores were identified in HC, target neural antigens, which is expected, as the analyzed symptoms are neuropsychological. This associatian was disturbed in both ME/CFS(+)FM and ME/CFS(-)FM groups – a number AAb targeting internal organ-specitic and non-tissue specific antigens correlated with the symptoms scores in these groups. Thus, our findings imply that ME/CFS and FM are organic multisystem diseases, rather than psychological disorder.

The pattern of correlations found in ME/CFS(-)FM (according to the manufacturer of the ELI-tests) suggests the role of the gut microbiom, disturbed detoxification mechanisms (namely, liver and kidney functioning) and an inflammatory process in the pelvic organs in the symptoms development. At the same time, the mechanisms, undelying the observed correlations, remains largely unclear and can differ between patients and healhty controls. For example, in HC anti-GABA-Re AAb were associated with lower mental component score, i.e. worse self-perceived mental health, while in patients from ME/CFS(-)FM group these AAb were inversly correlated with depression.

Changes in immunereactivity to β Endorphin were associated with more pronounced fatigue in both ME/CFS(-)FM and ME/CFS(+)FM groups, but not in HC. These findings suggest the dysfunction of endogenous opioid system in ME/CFS and FM. Interstingly, that a significant factor that differentiates β-endorphin from other endogenous opiates is its high affinity for and lasting effect on μ-opioid receptors[28]. When comparing patient groups and HC with regard to the associations of bodily pain with the studied AAb, it can be noted that patients from both groups abrogated the normal interconnection between anti-μ-Opioid-Re AAb and pain intensity. It was shown by Schrepf et al. in the study employed PET and fMRI together that dysregulation of the endogenous opiate system in FM could lead to less excitation in antinociceptive brain regions by incoming noxious stimulation, resulting in the hyperalgesia and allodynia commonly observed in this population[26].

Increasing evidence suggests that GFAP might be a biomarker for a number of neurological conditions, which is characterized by strong brain-specificity and high expression levels to the brain. Brain injury causes the release of GFAP and its breakdown products from injured astrocytes to the extracellular space, where these proteins equilibrate into the subarachnoid CSF compartment, then release to the circulating blood by glymphatic pathway or by diffusing pass the (possibly compromised) brain blood barrier[29]. It has been also reported that GFAP can serve as autoantigen, triggering AAb response in a subset of patients[29]. In our study changes in anti-GFAP AAb level were positevely associated with worse mental component score in ME/CFS(+)FM group and with illness duration in ME/CFS(-)FM group.

The network-based analyses has been recently implemented in the study of physiological autoimmunuty and its disturbances. In particular, distinct signatures of anti-GPCR AAb in HC, which were influenced by age, gender, and various diseases have been revealed[9].

Here, we determined the correlation signatures of some AAb targeting neural, internal organ-specific and non-organ-specific autoantigens in health and disease based on enzyme-linked immunosorbent assay (ELISA). AAB to β2-GP, Fc-IgG, CoM, LuM-02, GaM-02 had obviously more associations with other AAb in ME/CFS(-)FM group than in HC. Danilenko et al[30] showed with the same method as in our study, that anti-β2-GP AAb were increased in ME/CFS patients, but not in healthy participants, that alluded the link between ME/CFS and antiphospholipid syndrome earlier suspected by Berg et al. in 1999[31].

ME/CFS(+)FM group was characterized by an increase of associations of anti-collagen AAb and anti-TrM-03 AAb with other AAb. It was been recently reported that 81% of patients with ME/CFS and/or FM met Brighton criteria for hypermobility syndrome and 18% met 2017 hypermobile Ehlers–Danlos syndrome criteria. Hypermobility scores significantly predicted symptom levels in these patietns[32]. Notably, that a high titer of AAb to type I collagen has been recently found in patients with undifferentiated connective tissue dysplasia and joint hypermobility[33]. Earlier such individuals also were demonstrated to be predisposed to anti-thyroid au-toimmunity[34]. Another feature of AAb correlation pattern in ME/CFS(+)FM group was the abolished intragroup correlations between anti-neural AAb.

Based on our observations, we assume that AAb are natural components of the immune system and may become dysregulated not only in classical autoimmune conditions, but also in FM and ME/CFS. This assumption is in accordance with the perception of the role of the immune system in homeostatic regulation beyond host defense.

Author Contributions: Conceptualization, L.C., V.R. and Y.S.; methodology, A.Pol., A.Pukh.; software, A. Pukh.; formal analysis, A. Pukh, V.R.; investigation, V.R., N.G., I.K.; resources, L.C., Y.S.; data curation, A. Pukh.; writing—original draft preparation, V.R.; writing—review and editing, L.C.; visualization, V.R.; supervision, L.P.; project administration, A.P., Y.S.; funding acquisition, L.C., Y.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Russian Science Foundation, grant number 22-15-00113, https://rscf.ru/project/22-15-00113/

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Saint-Petersburg State University (protocol code 115-02-5 of 25-06-2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable

Acknowledgments: We authors would like to express their deepest gratitude to the patients suffered form ME/CFS and post-COVID-19 condition and found the strength to take part in the study (especially in the very beginning, when few specialists recognize the importance of the studied conditions), and provided new ideas for research in the area

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

[1] Goebel A, Krock E, Gentry C, Israel MR, Jurczak A, Urbina CM, et al. Passive transfer of fibromyalgia symptoms from patients to mice. J Clin Invest 2021;131. doi:10.1172/JCI144201.

[2] Krock E, Morado-Urbina Msc CE, Menezes Msc J, Hunt Phd MA, Sandström Phd A, Kadetoff D, et al. Fibromyalgia patients with high levels of anti-satellite glia cell IgG antibodies present with more severe symptoms. BioRxiv 2022:2022.07.06.498940. doi:10.1101/2022.07.06.498940.

[3] Rivera MC, Mastronardi C, Silva-Aldana CT, Arcos-Burgos M, Lidbury BA. Myalgic encephalomyelitis/chronic fatigue syndrome: A comprehensive review. Diagnostics 2019;9:91. doi:10.3390/diagnostics9030091.

 doi:10.20944/preprints202212.0224.v1

[4] Natelson BH. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Fibromyalgia: Definitions, Similarities, and Differences. Clin Ther 2019;41:612–8. doi:10.1016/j.clinthera.2018.12.016.

[5] Ramírez-Morales R, Bermúdez-Benítez E, Martínez-Martínez LA, Martínez-Lavín M. Clinical overlap between fibromyalgia and myalgic encephalomyelitis. A systematic review and meta-analysis. Autoimmun Rev 2022;21. doi:10.1016/J.AUTREV.2022.103129.

[6] Komaroff AL. Advances in Understanding the Pathophysiology of Chronic Fatigue Syndrome. JAMA 2019;322:499–500. doi:10.1001/jama.2019.8312.

[7] Missailidis D, Annesley SJ, Fisher PR. Pathological Mechanisms Underlying Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Diagnostics 2019;9. doi:10.3390/DIAGNOSTICS9030080.

[8] Freitag H, Szklarski M, Lorenz S, Sotzny F, Bauer S, Philippe A, et al. Autoantibodies to vasoregulative g-protein-coupled receptors correlate with symptom severity, autonomic dysfunction and disability in myalgic encephalomyelitis/chronic fatigue syndrome. J Clin Med 2021;10. doi:10.3390/JCM10163675/S1.

[9] Cabral-Marques O, Marques A, Giil LM, De Vito R, Rademacher J, Günther J, et al. GPCR-specific autoantibody signatures are associated with physiological and pathological immune homeostasis. Nat Commun 2018;9:5224. doi:10.1038/s41467-018-07598-9.

[10] Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The Chronic Fatigue Syndrome: A Comprehensive Approach to Its Definition and Study. Ann Intern Med 1994;121:953. doi:10.7326/0003-4819-121-12-199412150-00009.

[11] Carruthers BM, Jain AK, De Meirleir KL, Peterson DL, Klimas NG, Lerner AM, et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. J Chronic Fatigue Syndr 2003;11:7–115. doi:10.1300/J092v11n01_02.

[12] Institute of Medicine. Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Washington, D.C.: National Academies Press; 2015. doi:10.17226/19012.

[13] Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Häuser W, Katz RL, et al. 2016 Revisions to the 2010/2011 fibromyalgia diagnostic criteria. Semin Arthritis Rheum 2016;46:319–29. doi:10.1016/j.semarthrit.2016.08.012.

[14] Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473–83. doi:10.1097/00005650-199206000-00002.

[15] Smets EMA, Garssen B, Bonke B, De Haes JCJM. The multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 1995;39:315–25. doi:10.1016/0022-3999(94)00125-O.

[16] Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale: An updated literature review. J Psychosom Res 2002;52:69–77. doi:10.1016/S0022-3999(01)00296-3.

[17] Poletaev A, Boura P. The immune system, natural autoantibodies and general homeostasis in healthand disease. Hippokratia 2011;15:295.

[18] Meier LA, Binstadt BA. The Contribution of Autoantibodies to Inflammatory Cardiovascular Pathology. Front Immunol 2018;9:911. doi:10.3389/FIMMU.2018.00911.

[19] Fukushima K, Tsujino K, Futami S, Kida H. Natural Autoantibodies in Chronic Pulmonary Diseases. Int J Mol Sci 2020, Vol 21, Page 1138 2020;21:1138. doi:10.3390/IJMS21031138.

[20] Pashnina IA, Krivolapova IM, Fedotkina T V., Ryabkova VA, Chereshneva M V., Churilov LP, et al. Antinuclear Autoantibodies in Health: Autoimmunity Is Not a Synonym of Autoimmune Disease. Antibodies 2021;10:9. doi:10.3390/antib10010009.

[21] Cohen IR. Biomarkers, self-antigens and the immunological homunculus. J Autoimmun 2007;29:246–9. doi:10.1016/J.JAUT.2007.07.016.

[22] Poletaev A, Osipenko L. General network of natural autoantibodies as immunological homunculus (Immunculus). Autoimmun Rev 2003;2:264–71. doi:10.1016/S1568-9972(03)00033-8.

[23] Poletaev AB, Churilov LP, Stroev YI, Agapov MM. Immunophysiology versus immunopathology: Natural autoimmunity in human health and disease. Pathophysiology 2012;19:221–31. doi:10.1016/j.pathophys.2012.07.003.

[24] Laborit H. The major mechanisms of stress. Methods Achiev Exp Pathol 1991;15:1–26.

[25] Cope H, Mann A, Pelosi A, David A. Psychosocial risk factors for chronic fatigue and chronic fatigue syndrome following presumed viral illness: a case-control study. Psychol Med 1996;26:1197–209. doi:10.1017/S0033291700035923.

[26] Baraniuk JN. Review of the Midbrain Ascending Arousal Network Nuclei and Implications for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), Gulf War Illness (GWI) and Postexertional Malaise (PEM). Brain Sci 2022, Vol 12, Page 132 2022;12:132. doi:10.3390/BRAINSCI12020132.

[27] Glassford JAG. The Neuroinflammatory Etiopathology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Front Physiol 2017;8:88. doi:10.3389/fphys.2017.00088.

[28] Clark A, Lowry P, Smyth DG. 60 YEARS OF POMC: Lipotropin and beta-endorphin: a perspective. J Mol Endocrinol 2016;56:T13–25. doi:10.1530/JME-16-0033.

[29] Yang Z, Wang KKW. Glial Fibrillary acidic protein: From intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci 2015;38:364. doi:10.1016/J.TINS.2015.04.003.

[30] Danilenko O V., Gavrilova NY, Churilov LP. Chronic Fatigue Exhibits Heterogeneous Autoimmunity Characteristics Which Reflect Etiology. Pathophysiol 2022, Vol 29, Pages 187-199 2022;29:187–99. doi:10.3390/PATHOPHYSIOLOGY29020016.

[31] Berg D, Berg LH, Couvaras J, Harrison H. Chronic fatigue syndrome and/or fibromyalgia as a variation of antiphospholipid antibody syndrome: An explanatory model and approach to laboratory diagnosis. Blood Coagul Fibrinolysis 1999;10:435–8. doi:10.1097/00001721-199910000-00006.

[32] Eccles JA, Thompson B, Themelis K, Amato ML, Stocks R, Pound A, et al. Beyond bones: The relevance of variants of connective tissue (hypermobility) to fibromyalgia, ME/CFS and controversies surrounding diagnostic classification: an observational study. Clin Med 2021;21:53–8. doi:10.7861/CLINMED.2020-0743.

[33] Babamuradova Z, Shodikulova G. Content of type i collagen antibodies and their association with clinical manifestations of undifferentiated connective tissue dysplasia. Eur Sci 2020;2–1:82–5.

[34] Churilov LP, Stroev YI, Serdyuk IY, Kaminova-Mudzhikova OM, Belyaeva IV, Gvozdetsky AN, et al. Autoimmune thyroiditis: Centennial jubilee of a social disease and its comorbidity. Pathophysiology 2014;21:135–45. doi:10.1016/j.pathophys.2013.11.002.