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N. Azcue, A. Prada, R. Del Pino, M. Acera, T. Fernández-Valle, N. Ayo-Mentxakatorre, T. Pérez-Concha, A. Murueta-Goyena, J. V. Lafuente, A. López de Munain, G. Ruiz Irastorza, L. Ribacoba, I. Gabilondo, B. Tijero-Merino & J. C. Gómez-Esteban

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Involvement of autoantibodies against G protein-coupled receptors in post-COVID condition and Chronic Fatigue Syndrome

Azcue, N[1]; Prada, Á.[2,3]; Del Pino, R.[1*]; Acera, M.[1]; Fernández-Valle, T.[1,4,5]; Ayo-Mentxakatorre, N.[1], Pérez-Concha, T.[4,6]; Murueta-Goyena, A.[1,5]; Lafuente, J.V.[5]; López de Munain, A. [6,7,8,9,10]; Ruiz Irastorza, G.[11,12]; Ribacoba, L.[13]; Gabilondo, I.[1,4,14]; Tijero-Merino, B.[1,4,6,10]; Gómez-Esteban, J.C.[1,4,5,6,10*].

1.Neurodegenerative Diseases Group, Biobizkaia Health Research Institute, Barakaldo, Spain.

2.Department of Immunology, Donostia University Hospital-OSAKIDETZA, San Sebastián, Spain.

3.Spanish Network for the Research in Multiple Sclerosis, San Sebastian, Spain.

4.Department of Neurology, Cruces University Hospital-OSAKIDETZA, Barakaldo, Spain.

5.Department of Neurosciences, University of the Basque Country UPV/EHU, Leioa, Spain.

6. Department of Medicine, School of Medicine, University of Deusto, Bilbao, Spain.

7.Department of Neurology, Donostia University Hospital-OSAKIDETZA, San Sebastián, Spain.

8.Department of Neurosciences, Biogipuzkoa Health Research Institute, San Sebastián, Spain.

9. Department of Neurosciences. University of the Basque Country UPV-EHU, San Sebastián, Spain.

10. CIBERNED-CIBER, Institute Carlos III, Madrid, Spain.

11.Autoimmune Diseases Research Unit, Biobizkaia Health Research Institute, Barakaldo, Spain.

12. Department of Autoimmune Diseases, Cruces University Hospital-OSAKIDETZA, Barakaldo, Spain.

13.Department of Internal Medicine, Cruces University Hospital, Barakaldo, Spain.

14.The Basque Foundation for Science, IKERBASQUE, Bilbao, Spain.

*Corresponding author:

Juan Carlos Gómez-Esteban, M.D., Ph.D., Servicio de Neurología, Hospital de Cruces, Barakaldo, Spain. E-mail: juancarlos.gomezesteban@osakidetza.eus

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Rocio Del Pino Sáez, Ph.D. Biobizkaia Health Research Institute, Barakaldo, Spain. E-mail: delpinorocio@gmail.com

ABSTRACT

Purpose: Post-COVID condition (PCC) and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) are chronic disorders marked by fatigue, autonomic dysfunction, and cognitive impairment. Autoantibodies (AAbs) targeting adrenergic and muscarinic receptors have been implicated in their pathophysiology. This study aimed to investigate the association between these AAbs, autonomic nervous system (ANS) function, and cognitive performance in PCC and ME/CFS.

Methods: We included 96 PCC patients, 59 ME/CFS patients, and 36 healthy controls (HCs). Plasma AAbs against α1, β1, β2 adrenergic and M1–M4 muscarinic receptors were measured via ELISA. ANS function was evaluated using COMPASS-31, Sudoscan, hemodynamic tests (deep breathing, Valsalva, tilt test), and heart rate variability. Cognitive domains assessed included attention, fluency, processing speed, memory, visuoconstruction, perception, and executive functions.

Results: ME/CFS patients had significantly higher β2 adrenergic AAb titers than PCC and HCs ($F_{2,186} = 3.15$, $p = 0.046$). PCC patients showed more borderline/pathological M3 muscarinic AAb results compared to HCs. β2 AAb levels correlated with increased autonomic symptoms in PCC ($r = 0.27$, $p = 0.048$) and sympathovagal imbalance in ME/CFS ($r = 0.45$, $p = 0.001$). In ME/CFS, M1, M3, and M4 AAb titers positively correlated with verbal and working memory performance.

Conclusion: Distinct AAb profiles in PCC and ME/CFS suggest potential differences in immunological mechanisms. β2 adrenergic receptor AAbs were associated with measures of autonomic dysfunction in PCC patients, and with sympathovagal parameters in ME/CFS patients. Muscarinic AAbs were correlated with cognitive performance in ME/CFS, supporting a potential role of these autoantibodies in

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autonomic and cognitive dysfunction. These findings support further investigation of AAbs as biomarkers and therapeutic targets.

Keywords: Adrenergic Receptor Antibodies; Dysautonomia; Muscarinic Receptor Antibodies; Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; Post-COVID Condition.

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Introduction

Post-COVID condition (PCC) and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) are complex and debilitating conditions characterized by severe physical and mental fatigue that are disproportionate to the level of exertion[1,2]. These syndromes share overlapping clinical features, including Autonomic Nervous System (ANS) dysregulation, cognitive impairment, muscle fatigue, and chronic pain. Despite growing awareness, the underlying mechanisms driving these conditions remain poorly understood, hindering effective diagnostic and therapeutic approaches[3–5].

Previous studies have suggested a possible autoimmune basis for both syndromes[6–9]. One prominent hypothesis posits that after the resolution of an acute infection the immune system may produce an exaggerated reaction to the pathogen. This hyperactivation leads to a cytokine storm, neuroinflammation, reactivation of latent infections, and subsequent immune deregulation[10–14]. Such processes may explain the persistence of symptoms, including fatigue and cognitive decline, long after the acute phase of infection has subsided. In particular, immune deregulation has been linked to the presence of autoantibodies (AAbs) targeting receptors critical to ANS functionality[15–18].

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Among the AAbs implicated in these syndromes are those targeting G protein-coupled receptors, such as adrenergic (AdR) and muscarinic acetylcholine receptors (AChR), which play crucial roles in regulating autonomic and cognitive functions[19,20]. Adrenergic receptors, located in both the central and peripheral nervous systems, are integral to controlling immune responses, energy metabolism, cardiac activity, and cognition[21]. Similarly, muscarinic receptors, also expressed in the central and peripheral nervous systems, are essential for neuromuscular function and neurological transmission[22]. Dysregulation of these receptors, whether through heightened or diminished activity

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of their corresponding AAbs, may contribute to the constellation of symptoms seen in PCC and ME/CFS.

Evidence from recent studies underscores the role of these autoantibodies in ANS dysfunction. For instance, elevated levels of AAbs targeting β1 and β2 AdR and M3 M4 muscarinic AChR have been observed in both ME/CFS and PCC patients[15,23–27]. These AAbs have been associated with a range of clinical manifestations, including fatigue, cognitive impairment, muscle pain, and dysautonomia.

While increasing evidence suggests substantial clinical and biological overlap between PCC and ME/CFS, PCC remains a heterogeneous condition that may include ME/CFS-like presentations as well as distinct immunological phenotypes. However, it remains unclear whether PCC and ME/CFS represent manifestations along a shared immunopathophysiological continuum or partially distinct conditions with overlapping symptomatology but divergent underlying mechanisms. Direct comparative studies evaluating autoantibody profiles in relation to objective autonomic and cognitive measures across both conditions are still limited. Clarifying both shared and condition-specific immunological associations is therefore essential to better understand whether these autoantibodies reflect common downstream consequences of post-infectious immune dysregulation or condition-specific mechanisms contributing to symptom expression.

Therefore, this study aimed to investigate the relationship between adrenergic and muscarinic AAbs, autonomic nervous system function, and cognitive performance in patients with PCC and ME/CFS, compared to healthy controls. By directly comparing these conditions, this study seeks to identify both shared and condition-specific immunological associations and to clarify the potential role of autoantibodies as biological correlates of autonomic and cognitive dysfunction in post-infectious syndromes. Ultimately, improving the biological characterization of these associations may contribute to a better

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understanding of disease mechanisms and help inform future mechanistic and therapeutic research.

Materials and methods

Participants and demographic data

A total of 59 patients with ME/CFS, 96 patients with PCC, and 36 healthy controls (HCs) were recruited, with general inclusion criteria requiring participants to be over 18 years old, have adequate comprehension and communication skills, and follow the technical instructions of the research team. The general exclusion criteria included severe illnesses that contraindicate participation, primary psychiatric disorders, autoimmune conditions explaining the symptoms, pregnancy or breastfeeding, severe trauma, substance abuse, use of pacemakers, and radiological diagnoses of structural brain pathology. Primary psychiatric disorders were ruled out during the recruitment process through clinical evaluation conducted by experienced neurologists, based on medical history and clinical interview. Specific criteria for the PCC group required a prior COVID-19 diagnosis confirmed by nasal PCR, detection of IgG/IgM antibodies against SARS-CoV-2, or a medical report, particularly for early-pandemic patients who could not undergo testing. PCC patients had to present subjective symptoms such as physical or mental fatigue, palpitations, sensory symptoms, or dysautonomic symptoms persisting for at least 12 weeks, with additional specific exclusion criteria including SARS-CoV-2 infections causing respiratory issues beyond 12 weeks post-infection or requiring ICU admission and/or severe bilateral pneumonia hospitalization with invasive measures. For the ME/CFS group, patients had to meet the diagnostic criteria proposed by Fukuda[28], which require severe fatigue disproportionate to activity lasting at least six months accompanied by at least four of the following symptoms: memory impairment, post-exertional malaise, unrefreshing sleep, arthralgia, headaches, sore throat, and/or lymph node tenderness.

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The study protocol was approved by the Basque Drug Research Ethics Committee [*Comité de Ética de la Investigación con medicamentos de Euskadi* (CEIm-E) PI2020210)]. All participants gave written informed consent prior to their participation in the study, in accordance with the tenets of the Declaration of Helsinki.

Autoantibodies

In this study, we focused on the analysis of AAbs targeting G-protein-coupled receptors. Specifically, AAbs against muscarinic acetylcholine receptors (AChR) M1–M4 and adrenergic receptors (AdR) α1, β1, and β2 (collectively referred to as Mx AChR AAbs and α/β AdR AAbs, respectively). The technique used to analyze AdR and AChR muscarinic AAbs in plasma was an enzyme-linked immunosorbent assay (ELISA) (CellTrend GmbH©, Luckenwalde, Germany). This method enables the detection and quantification of specific autoantibodies by using antigen-coated plates, where the AAbs in the plasma bind to these antigens. A secondary enzyme-conjugated antibody is then added, allowing detection via a color change. The intensity of the color change is measured with a spectrophotometer, enabling the determination of AAbs concentration. ELISA-based assays detect antibody binding but do not directly assess functional receptor activity. Therefore, these measurements do not distinguish between agonistic, antagonistic, or neutral functional effects.

Blood samples were collected in EDTA tubes for the analysis of AAbs in plasma. The samples were processed by centrifugation at 20°C at 3599 rpm for 15 minutes to separate the plasma. The plasma was aliquoted and frozen at -40°C. Blood samples from all participants were collected and processed within 1 hour.

Pathological levels were defined according to the reference cut-off values provided by the manufacturer (CellTrend GmbH), based on their internal validation datasets. These thresholds are assay-specific and were not derived from the present study population. Reference values and pathological cut-off thresholds are available for α1, β1, β2, M3, and

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M4-targeting AAbs. However, no established reference ranges or pathological cut-off values are provided by the manufacturer for M1 and M2 autoantibodies.

Autonomic function assessment

A comprehensive evaluation of the ANS was conducted for all participants, covering autonomic symptoms, sudomotor, and hemodynamic autonomic function. Autonomic symptoms were assessed using the Composite Autonomic Symptom Score-31 (COMPASS-31), a validated tool that measures symptoms across six domains of autonomic function.

Sudomotor function, regulated by the sympathetic cholinergic system, was evaluated non-invasively using the Sudoscan device (Impeto Medica Paris France). This test quantified the electrochemical skin conductance (ESC) on the palms and soles, expressing results in microSiemens (µS).

Hemodynamic autonomic function was measured using the Task Force Monitor (CNSystems© Graz, Austria) under standardized conditions (including stable room temperature). The Task Force Monitor is a validated, non-invasive system for comprehensive hemodynamic and autonomic assessment, demonstrating high accuracy and reliability across multiple cardiovascular parameters[29]. Resting heart rate (HR) and interbeat intervals were recorded while participants lay supine for 10 minutes. Baroreflex sensitivity (BRS) was assessed via the sequence method to evaluate autonomic cardiovascular regulation. High-frequency normalized units of R-R interval variability (HFnu-RRI) were used as an index of parasympathetic activity, while low-frequency spectral power normalized units of diastolic blood pressure (LFnu-dBP) was analyzed to reflect sympathetic vascular modulation.

Parasympathetic function was assessed through specific tests. In the deep breathing test, participants performed six cycles of deep breathing (five seconds for each inspiration and expiration) while at

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rest. The difference between the maximum and minimum HR during each cycle, known as the E/I ratio, served as a sensitive and specific indicator of cardiovagal function. The Valsalva maneuver, performed by exhaling at 40 mmHg for 15 seconds, was used to assess both adrenergic and parasympathetic function. The Valsalva ratio, calculated as the maximum HR in phase III divided by the minimum HR in phase IV, provided key insights into the relationship between HR and blood pressure changes. The systolic blood pressure recovery time (PRT) after phase III was also measured as an indicator of adrenergic function. The tilt test was performed with passive tilt at a 60° angle for at least 10 minutes to assess orthostatic intolerance. All tests were conducted by experienced neurologists to ensure accuracy and reliability.

Cognitive and neuropsychiatric assessment

A comprehensive neuropsychological evaluation was conducted (see Supplementary Material for detailed description and references of all tests), encompassing both general cognitive status as a screening measure and the following cognitive domains: attention span, sustained attention, semantic and phonological verbal fluency, processing speed, verbal and visual memory, visuoconstructive ability, visual perception, and executive functions.

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Statistical analysis

Statistical analyses were carried out with IBM SPSS Statistics for Windows, 26.0 version (IBM SPSS, Armonk, NY, USA). The assumptions of normality and homogeneity of variances of the variables were analyzed. All results were transformed into *z*-scores, creating composites for the different cognitive domains. For consistency, variables where higher scores indicate worse performance were inverted prior to standardization. Specifically, we inverted completion times for the Trail Making Test A and B (TMT-A and TMT-B), as well as error scores from the Modified Wisconsin Card Sorting Test (M-WCST) and the Toulouse-Piéron Revised (TP-R) test. Group differences for

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continuous variables were analyzed using analysis of covariance (ANCOVA), adjusting for relevant covariates according to the outcome of interest. For analyses involving autoantibody levels, age and sex were included as covariates given their potential influence on immune responses and autoantibody profiles. For cognitive outcomes, age and educational level were included as covariates because of their established and more direct association with cognitive performance. Categorical variables were analyzed using the Chi-square test. The correlations between variables were analyzed using the bivariate Pearson statistic. Pairwise comparisons between groups were performed based on estimated marginal means, and corresponding p-values are reported. In addition, a principal component analysis (PCA) was conducted using the AAb levels to explore underlying patterns of autoantibody distribution. Finally, stepwise linear regressions were performed to observe how AAbs levels influenced cognitive performance and autonomic response. Statistical significance was set at $p < 0.05$ (two-tailed).

This study was designed as an exploratory analysis, and no formal a priori sample size calculation was performed. Given the exploratory nature of the study, no correction for multiple comparisons was applied, and results should therefore be interpreted with caution. In addition, group sizes were not fully balanced due to recruitment constraints and the availability of well-characterized patients fulfilling strict diagnostic criteria.

Results

Demographic and clinical data

The mean age across the groups shows similarities, with PCC at 45.54 years, ME/CFS at 44.25 years, and HCs at 42.31 years. The proportion of females was highest in the ME/CFS group (94.9%), followed by HCs (77.8%), and PCC (74.0%). The results of autoantibody titers, hemodynamic and ANS parameters, as well as cognitive performance

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outcomes from the neuropsychological battery, are presented in Table 1.

Immunological profile

No significant differences were found in the titers of AAbs against muscarinic AChR or AdR α1 and β1 between groups. However, differences were observed for AAbs targeting β2 AdR, with the analysis adjusted for age and sex ($F_{2,186}$= 3.15, p=0.046). Patients with ME/CFS exhibited higher titers of this AAb compared to PCC patients and HCs (Fig 1).

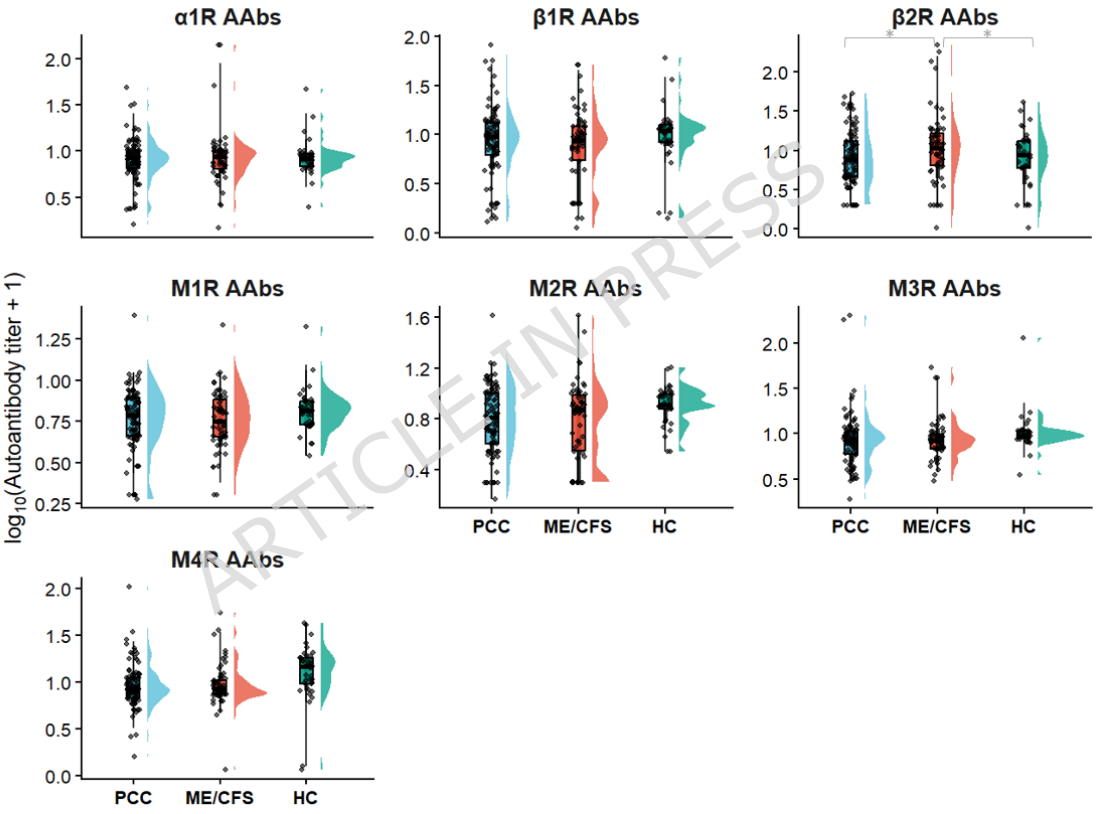

Fig 1. AAb levels per group. *p≤0.05. AAbs: autoantibodies. Raincloud plots showing the distribution of serum autoantibody titers against α1-adrenergic (α1R), β1-adrenergic (β1R), β2-adrenergic (β2R), and muscarinic M1–M4 receptors (M1R–M4R) in patients with post-COVID condition (PCC), myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), and healthy controls (HC). Individual data points represent participants, boxplots indicate median and interquartile range, and half-violin plots illustrate the data distribution. Titers are

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displayed on a log10 scale (log10[titer + 1]) to improve visualization of skewed distributions.

In addition to the comparisons in AAb levels, the percentage of participants with pathological results was also analyzed for those AAb with defined pathological thresholds. Pathological levels of AAb against M3 AChR were found in 19.8% of PCC patients, 18.6% of ME/CFS patients, and 16.7% of HCs.

Pathological levels of AAb were found in PCC patients for M4 AChR (21.9%), α1 (56.3%), β1 (15.6%), and β2 AdR (21.5%). In ME/CFS patients, pathological results were observed for M4 AChR (18.6%), α1 (55.9%), β1 (16.9%), and β2 AdR (27.5%). Among HCs, 58.3% showed pathological levels for M4 AChR and α1 receptors, 8.3% for β1, and 13.9% for β2 AdR.

When analyzing percentage differences between groups, statistically significant differences were observed in AAbs against M3 AChR (χ^2=12.48, p=0.014), with differences between PCC patients and HCs (χ^2=11.78, p=0.003), as well as between the ME/CFS group and HCs (χ^2=10.28, p=0.006).

Additionally, significant differences were observed in the percentages of pathological and normal results for AAbs against M4 AChR (χ^2=20.89, $p \leq 0.001$), with differences between PCC patients and HCs (χ^2=16.04, $p \leq 0.001$), as well as between ME/CFS patients and HCs (χ^2=15.77, $p \leq 0.001$).

A PCA was conducted using z-scores of AAb levels targeting M1–M4 AChR, α1, β1, and β2 AdR. The first two principal components explained a total of 69.8% of the variance (PC1 = 46.7%, PC2 = 23.1%). Although the analysis captured underlying patterns in AAbs distribution, no clear separation between PCC, ME/CFS, and HCs was observed. This suggests that the AAb profiles, while variable, do not significantly differentiate between the clinical groups in this dataset. Thus, PCA did

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not reveal a distinct clustering pattern, suggesting limited discriminatory power in this dataset.

Implication of AAbs in the ANS

Regarding the ANS assessment, 35.6% of ME/CFS patients and 14.6% of PCC patients met the criteria for POTS. Based on this, potential differences in AAb titers were analyzed by dividing both ME/CFS and PCC groups into patients with and without POTS. However, no statistically significant differences were found between these subgroups. Nonetheless, several statistically significant correlations were observed between AAbs and autonomic variables.

In PCC patients, higher levels of AAbs against the β2 AdR were correlated with increased dysautonomic symptoms as measured by COMPASS-31 ($r=0.27$, $p=0.048$). Higher levels of AAbs against the M3 AChR were associated with a higher sympathovagal index ($r=0.26$, $p=0.012$).

Stepwise linear regressions were performed to assess the involvement of different AAbs in the autonomic and hemodynamic response of patients (Table 1). In PCC patients, AAbs against M4 AChR accounted for 8.9% of baroreflex sensitivity, along with age, which explained 6.3% ($F_{2,186}=5.31$, $p=0.008$). HFnu-RRI was explained by AAbs against M4 AchR in 12.3% and by sex in 9.5% ($F_{2,186}=8.21$, $p\leq0.001$). The variability of LF-dBP was explained by AAbs against M4 AChR in 30.0% ($F_{2,186}=25.67$, $p\leq0.001$).

In ME/CFS patients, higher levels of AAbs against the M1 AChR were associated with lower systolic blood pressure in phase 4 of the Valsalva maneuver. Additionally, AAbs against the β2 AdR correlated with a higher sympathovagal index ($r=0.45$, $p=0.001$). In these patients, stepwise linear regressions revealed that AAbs against M1 AChR accounted for 21.0% of the difference in HR from supine to passive tilt, along with AAbs against B1 AdR, which explained 9.8% ($F_{4,186}=7.36$, $p=0.002$).

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In HCs, a higher HR during the passive tilt test was associated with elevated levels of AAbs against M3 AChR (r=0.43, p=0.009), α1 (r=0.46, p=0.005), and β1 AdR (r=0.52, p=0.001). Additionally, greater differences between supine HR and HR during the tilt test correlated with higher levels of AAbs against M3R (r=0.61, p<0.001), M4R (r=0.35, p=0.039), α1 (r=0.65, p<0.001), β1 (r=0.67, p<0.001), and β2 AdR (r=0.53, p<0.001). Additionally, higher levels of AAbs against M1 AChR correlated with an increased sympathetic-vagal index (r=0.39, p=0.020), as well as reduced ESC in both palms (r=-0.44, p=0.011) and soles (r=-0.57, p≤0.001). In HCs, AAbs against β1 AdR accounted for 24.8% of the variance in HR during the tilt test ($F_{4,186}$=12.19, p≤0.001). Furthermore, these same AAbs explained 43.9% of the difference in HR from the supine position to passive standing ($F_{4,186}$=27.56, p<0.001).

Implication of AAbs in cognition

Regarding the impact of these AAbs on cognitive performance, no significant correlations were observed between AAb titers and cognitive performance in PCC patients. However, in ME/CFS patients, better verbal memory performance was correlated with higher levels of AAbs against M1 (r=0.33, p=0.013), M3 (r=0.33, p=0.011), M4 AChR (r=0.32, p=0.016), and α1 AdR (r=0.33, p=0.013). Additionally, better working memory performance was observed in patients with higher levels of AAbs against M1 (r=.49, p<0.001), M2 (r=0.40, p=0.002), M3 (r=0.29, p=0.031), and M4 AChR (r=.34, p=0.011). In HCs, higher levels of AAbs against M4 AChR were significantly correlated with better performance in verbal memory tasks (r=.39, p=0.018).

The relationship between these AAbs and cognitive performance was also examined, adjusting the models for age and educational level. In patients with PCC, AAbs against M3R accounted for 17.2% of cognitive performance in verbal memory and 10.0% in visual memory. In patients with ME/CFS, verbal memory performance was explained by AAbs against M1 AChR (16.7%), α1 (6.4%), and β2 AdR (6.9%) (F=11.05,

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$p \leq 0.001$). Furthermore, in this group, working memory was associated with AAbs against the muscarinic M1 AChR (26.8%) (Fig 2), while visuospatial ability was explained by AAbs against the β2 AdR (13.3%) ($F_{2,186}=6.74$, $p=0.013$). In HCs, verbal memory was explained by M4 AChR AAbs in 15.7% and by α1R AAbs in 12.0% ($F_{2,186}=6.15$, $p=0.015$).

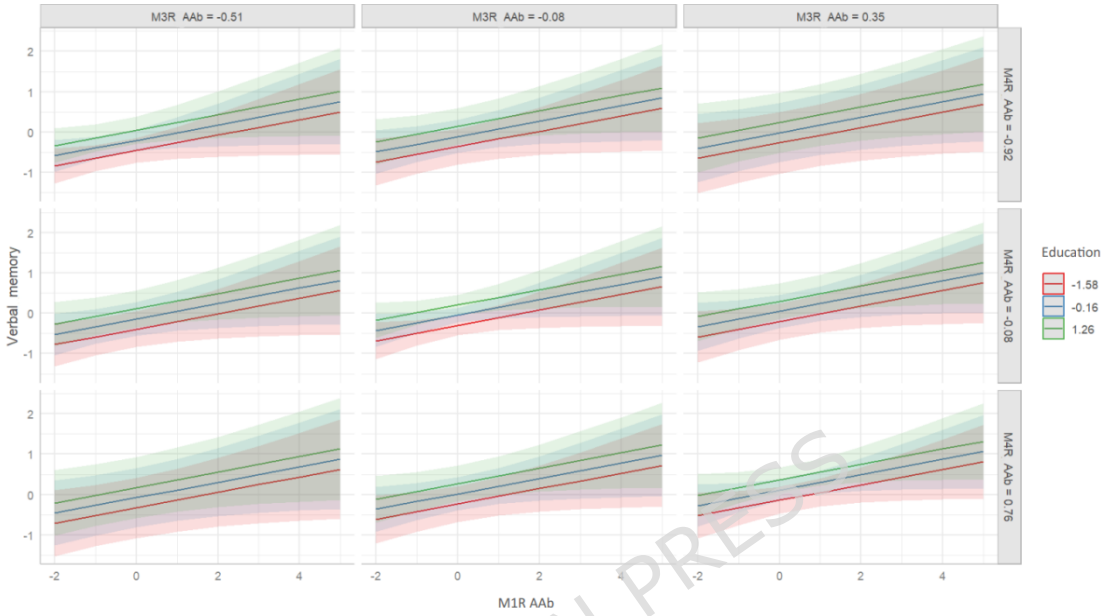

Fig 2. Implication of muscarinic AAbs in verbal memory in patients with ME/CFS. The values are shown in Z-scores. AAb: autoantibodies; ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome.

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Discussion

This study aimed to investigate the potential autoimmune nature of PCC and ME/CFS by analyzing the relationship between muscarinic AChR and AdR AAbs, ANS functionality, fatigue level, and cognitive impairment. Our results suggest an association between adrenergic receptor autoantibodies and autonomic dysfunction in ME/CFS, with significantly higher β2 adrenergic receptor autoantibody levels observed in ME/CFS patients. Additionally, the proportion of participants with pathological AAb levels varied across groups, with PCC patients showing the highest percentage of borderline and pathological results for M3 AChR AAbs. These findings highlighted

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distinct immunological profiles in PCC and ME/CFS, supporting a potential role of AAbs in cognitive and autonomic dysfunction in both conditions. Although group-level AAb profiles did not clearly distinguish between our patients, the observed associations with cognitive and autonomic outcomes suggested functional relevance of these autoantibodies beyond diagnostic classification. Importantly, absolute differences in AAb titers between groups were modest, and substantial overlap was observed across PCC, ME/CFS, and HCs. These findings highlight the need for further studies to clarify their biological and clinical significance.

AAbs against β2-adrenergic receptors were associated with markers of increased sympathetic activation in ME/CFS patients, suggesting that POTS or resting tachycardia in this group is not solely due to AdR or muscarinic AChR AAbs, as reported in other studies. Unlike previous findings, HCs in our sample showed a relationship between higher AAb levels and increased heart rate during passive standing. Additional mechanisms, such as elevated catecholamines or peripheral sympathetic denervation causing central hypovolemia, may also play a role. Measuring catecholamine levels at rest and during orthostatic stress could help clarify POTS pathophysiology in these patients[30].

M1 AChR plays a critical role in processes like synaptic plasticity, long-term potentiation, and memory, particularly in brain regions like the hippocampus and prefrontal cortex[33,34]. Contrary to expectations, higher AAb titers, especially against M1 AChR, were associated with better cognitive performance in verbal and working memory in ME/CFS patients. One possible explanation is that these autoantibodies may modulate cholinergic signaling in a compensatory manner, potentially enhancing receptor function under conditions of baseline cholinergic dysregulation. Similar modulatory effects have been described for other GPCR-targeting autoantibodies, which can bind to extracellular receptor domains and influence receptor activity through allosteric mechanisms[35]. Alternatively, these associations may reflect underlying

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alterations in muscarinic receptor regulation in ME/CFS, rather than a direct functional benefit of the autoantibodies themselves. Anti-M1R AAbs may also reflect adaptive responses to cholinergic or autonomic imbalance observed in these patients[27,36,37]. These findings should be interpreted cautiously. Autoantibodies targeting GPCRs may exert diverse functional effects depending on their properties and biological context, and their presence does not necessarily imply a direct pathogenic or protective role. Importantly, the observed associations represent correlations and do not establish causality. Furthermore, ELISA-based assays detect antibody binding but do not assess functional receptor activity, and therefore cannot determine whether these autoantibodies exert agonistic, antagonistic, or modulatory effects. Functional assays will be required to clarify their potential pathogenic, compensatory, or epiphenomenal roles[31,32].

The study has certain limitations. First, the sample was not matched for age and sex, although statistical analyses were adjusted to mitigate potential biases arising from these differences. Second, the diagnostic criteria used for ME/CFS were based on the Fukuda et al. definition. Although alternative criteria exist, such as the Canadian Consensus or International Consensus Criteria, the Fukuda criteria were selected due to their widespread use in research, facilitating comparison with previous studies and ensuring broader participant inclusion. Another limitation is related to the diagnostic criteria used for PCC. In a subset of early-pandemic patients, diagnosis was based on clinical presentation and medical reports without laboratory confirmation of SARS-CoV-2 infection. Although this reflects real-world clinical practice during the early phases of the pandemic, it may introduce a risk of misclassification and should be considered when interpreting the results. Another important limitation is that medication use was not systematically controlled for in the analyses. Pharmacological treatments such as beta-blockers, antidepressants, or other centrally acting agents may influence autonomic function, cognitive performance, and potentially immune responses, and thus may

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represent relevant confounding factors. In addition, PCC represents a clinically heterogeneous condition with potentially diverse underlying mechanisms, which may contribute to variability in immunological and physiological findings. Furthermore, the cross-sectional design limits the ability to determine temporal or causal relationships between AAb levels and autonomic or cognitive outcomes, and the observed associations do not establish causality or necessarily reflect direct pathogenic mechanisms. Therefore, these findings should be considered exploratory and hypothesis-generating. Future studies including larger and more homogeneous cohorts, longitudinal designs, and detailed clinical and treatment characterization will be necessary to better understand the variability, functional significance, and potential biological relevance of these AAbs.

Conclusion

Our findings emphasize the importance of recognizing ME/CFS and PCC as complex, multi-systemic disorders in which the immune, and nervous systems are intricately interconnected. These results indicate distinct immunological profiles in both conditions, reinforcing the role of AAbs in autonomic and cognitive dysfunction. Specifically, β2 adrenergic receptor AAbs were associated with dysautonomia, while muscarinic AAbs may modulate cognitive performance in ME/CFS, suggesting a potential modulatory role in cholinergic signaling.

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Conflict of Interest: Nothing to report.

Data availability: Anonymized data not published within this article will be made available by request from any qualified investigator.

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Abbreviations: AAbs: autoantibodies; AChR: acetilcholine receptor; AdR: adrenergic receptor; ANS: Autonomic Nervous System; BRS: Baroreflex sensitivity; ESC: electrochemical skin conductance; HCs: healthy controls; HFnu-RRI: normalized units of R-R interval variability; LFnu-dBP: normalized units of diastolic blood pressure; ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; PCA: principal component analysis; PCC: post-COVID condition.

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	PCC M (SD)	ME/CFS M (SD)	HCs M (SD)	Statistics	Post hoc comparisons (EMM-adjusted p-values)		
					PCC vs HCs	ME/CFS vs HCs	PCC vs ME/CFS
Age, years	45.54 (9.57)	44.25 (10.27)	42.31 (9.88)	F=1.99			
Female, *n* (%)	71 (74.0%)	56 (94.9%)	28 (77.8%)	χ^2= 7.77*			0.006
Years of education	16.23 (3.36)	15.11 (5.45)	16.79 (2.72)	F=2.61			
COMPASS 31	19.77 (9.16)	25.98 (11.02)	3.75 (4.63)	F=85.86***	≤0.001	≤0.001	≤0.001
MFIS	63.61 (15.01)	66.69 (14.81)	10.27 (11.74)	F=292.22***	≤0.001	≤0.001	
Immunology							
M1R Aab	5.32 (2.90)	5.26 (2.94)	5.91 (2.87)	F = 1.20			
M2R Aab	6.42 (5.14)	6.89 (6.95)	7.56 (2.74)	F = 1.35			
M3R Aab	11.70 (26.53)	9.28 (8.77)	11.75 (17.69)	F = 0.03			
M4R Aab	9.87 (11.04)	9.28 (8.77)	11.75 (17.69)	F = 2.52			
α1RAab	8.32 (6.22)	12.35 (24.88)	8.67 (7.23)	F = 1.29			
β1R Aab	10.84 (12.21)	9.93 (10.70)	11.11 (10.09)	F = 0.18			
β2R Aab	11.35 (11.94)	22.92 (43.62)	9.57 (8.52)	F = 3.15*	0.031	0.033	
Autonomic Nervous System							
Palms ESC (μS)	71.42 (16.40)	66.23 (16.21)	72.68 (10.38)	F=2.29			
Soles ESC (μS)	74.77(13.07)	70.53 (14.53)	70.65 (13.81)	F=0.67			
HFnu-RRI (%)	37.71 (16.36)	36.38 (16.29)	41.85 (15.84)	F=1.24			
LFnu-dBP (%)	45.59 (14.69)	44.96 (14.78)	44.33 (12.90)	F=0.39			
LF/HF	2.25 (2.47)	3.24 (7.48)	1.50 (0.98)	F=2.46			
SV	76.72 (16.67)	76.63 (19.27)	81.28 (17.86)	F=4.27*		0.004	
BRS mean	13.28 (7.78)	12.67 (9.55)	14.49 (8.42)	F=2.39	0.040		
Deep breathing index	17.01 (7.91)	15.79 (7.54)	17.02 (7.63)	F=0.09			
E/I ratio	1.27 (0.13)	1.26 (0.14)	3.64 (13.80)	F=1.72			
Valsalva ratio	1.06 (0.20)	1.10 (0.24)	1.06 (0.18)	F=0.84			
Valsalva PRT (s)	2.49 (1.04)	2.80 (1.03)	2.52 (0.56)	F=1.50			
ΔsBP phase II late	-12.55 (24.14)	-12.18 (18.39)	-7.20 (21.67)	F=2.30	0.049		
ΔsBP phase IV	22.32 (19.44)	18.70 (17.19)	21.62 (17.35)	F=1.05			
Hemodynamic responses to Tilt							
Supine							
sBP	113.11 (13.50)	111.71 (17.30)	110.91 (17.30)	F=0.05			
dBP	74.73 (12.74)	74.62 (12.17)	72.85 (14.28)	F=0.16			

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HR	73.28(12.14)	77.33 (13.92)	66.71 (9.63)	F=13.26***	≤0.001	≤0.001	≤0.001
Mean values (Tilt)							
sBP	129.88 (41.89)	126.15 (37.61)	125.39 (19.90)	F=0.05			
dBP	88.79 (26.40)	86.15 (14.49)	86.84 (18.34)	F=0.16			
HR	84.19 (14.98)	90.45 (15.78)	75.70 (11.36)	F=13.26***	≤0.001	≤0.001	0.039
Cognition							
General cognition	-0.18 (0.91)	-0.21 (1.18)	0.66 (0.43)	F=8.36***	≤0.001	≤0.001	≤0.001
Verbal fluency	-0.22 (0.82)	-0.24 (0.80)	0.86 (0.69)	F=25.35***	≤0.001	≤0.001	≤0.001
Processing speed	-0.18 (0.45)	-0.36 (0.60)	0.01 (0.28)	F=31.56***	≤0.001	≤0.001	
Attention	-0.14 (0.77)	-0.35 (0.73)	0.60 (0.80)	F=17.28***	0.005	≤0.001	
Verbal memory	-0.18 (0.87)	-0.05 (0.74)	0.53 (0.61)	F=15.95***	≤0.001	≤0.001	
Visual memory	-0.02 (0.79)	-0.18 (0.81)	0.24 (0.67)	F=1.94***	0.048	0.002	
Visuoconstructive ability	-0.03 (0.87)	-0.14 (1.24)	0.22 (0.88)	F=2.47*		0.010	
Visuospatial perception	0.03 (1.08)	-0.26 (0.95)	0.29 (0.73)	F=7.86***			
Executive functions	-0.05 (0.69)	-0.22 (0.64)	0.39 (0.53)	F=19.72***	≤0.001	≤0.001	

Table 1. Demographic and clinical data

*$p \leq .05$; ** $p \leq .01$; ***$p \leq .001$. *Note: Cognitive domains are shown in z-scores. The ANCOVA statistic was used, with sex, age and education as covariates cognitive domains' comparisons.* COMPASS: The Composite Autonomic Symptom Score; EMM: Estimated marginal means; HCs: healthy controls; ESC: electrochemical skin conductance; ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; MFIS: Modified Fatigue Impact Scale; PCC: post-COVID condition.

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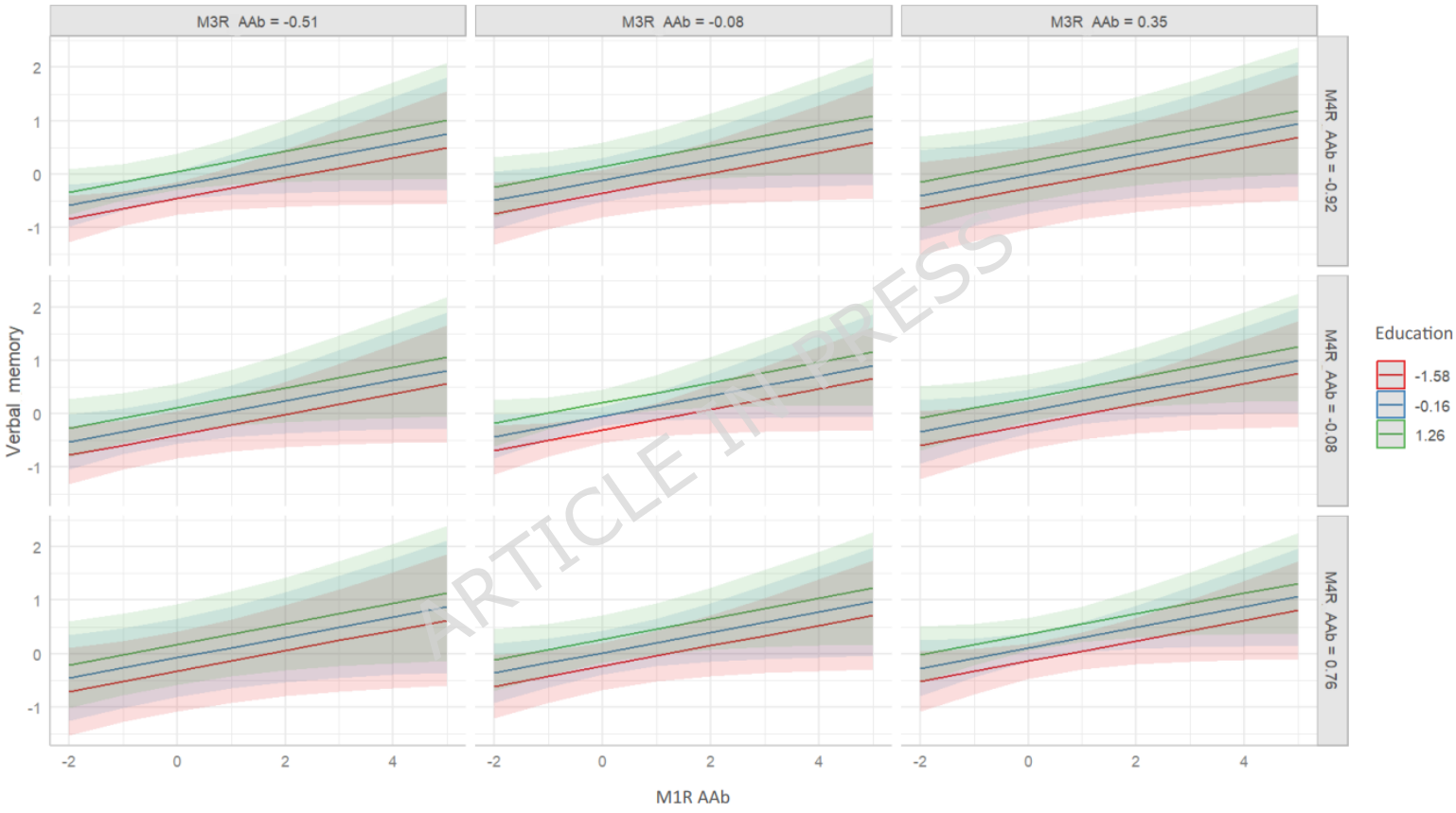

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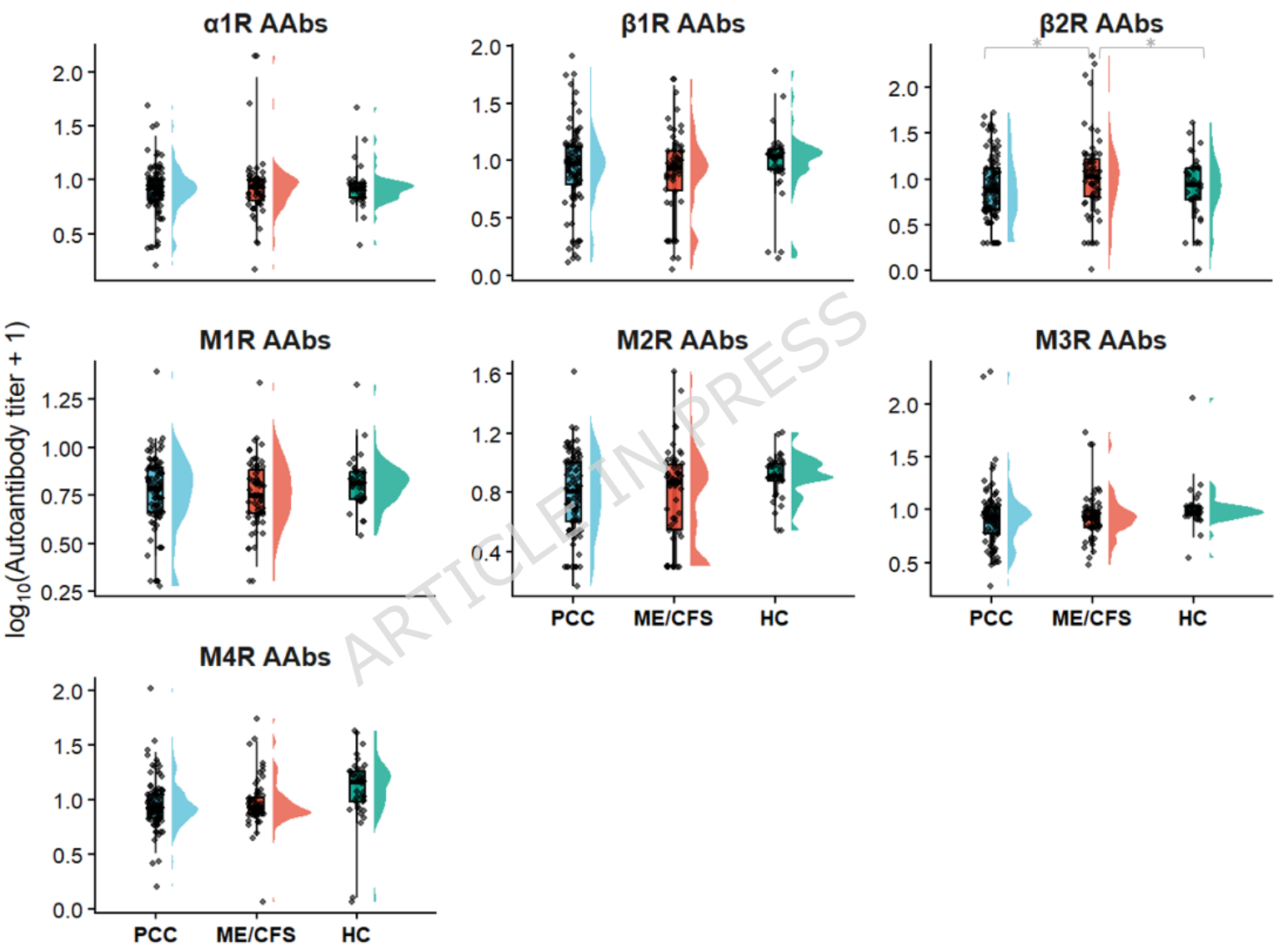

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Years of education	16.23 (3.36)	15.11 (5.45)	16.79 (2.72)	F=2.61			
COMPASS 31	19.77 (9.16)	25.98 (11.02)	3.75 (4.63)	F=85.86***	≤0.001	≤0.001	≤0.001
MFIS	63.61 (15.01)	66.69 (14.81)	10.27 (11.74)	F=292.22***	≤0.001	≤0.001	
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M1R Aab	5.32 (2.90)	5.26 (2.94)	5.91 (2.87)	F = 1.20			
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β2R Aab	11.35 (11.94)	22.92 (43.62)	9.57 (8.52)	F = 3.15*	0.031	0.033	
Autonomic Nervous System							
Palms ESC (µS)	71.42 (16.40)	66.23 (16.21)	72.68 (10.38)	F=2.29			
Soles ESC (µS)	74.77(13.07)	70.53 (14.53)	70.65 (13.81)	F=0.67			
HFnu-RRI (%)	37.71 (16.36)	36.38 (16.29)	41.85 (15.84)	F=1.24			
LFnu-dBP (%)	45.59 (14.69)	44.96 (14.78)	44.33 (12.90)	F=0.39			
LF/HF	2.25 (2.47)	3.24 (7.48)	1.50 (0.98)	F=2.46			
SV	76.72 (16.67)	76.63 (19.27)	81.28 (17.86)	F=4.27*		0.004	
BRS mean	13.28 (7.78)	12.67 (9.55)	14.49 (8.42)	F=2.39	0.040		
Deep breathing index	17.01 (7.91)	15.79 (7.54)	17.02 (7.63)	F=0.09			
E/I ratio	1.27 (0.13)	1.26 (0.14)	3.64 (13.80)	F=1.72			
Valsalva ratio	1.06 (0.20)	1.10 (0.24)	1.06 (0.18)	F=0.84			
Valsalva PRT (s)	2.49 (1.04)	2.80 (1.03)	2.52 (0.56)	F=1.50			
ΔsBP phase II late	-12.55 (24.14)	-12.18 (18.39)	-7.20 (21.67)	F=2.30	0.049		
ΔsBP phase IV	22.32 (19.44)	18.70 (17.19)	21.62 (17.35)	F=1.05			
Hemodynamic responses to Tilt							
Supine							
sBP	113.11 (13.50)	111.71 (17.30)	110.91 (17.30)	F=0.05			
dBP	74.73 (12.74)	74.62 (12.17)	72.85 (14.28)	F=0.16			
HR	73.28(12.14)	77.33 (13.92)	66.71 (9.63)	F=13.26***	≤0.001	≤0.001	≤0.001

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Mean values (Tilt)							
sBP	129.88 (41.89)	126.15 (37.61)	125.39 (19.90)	F=0.05			
dBP	88.79 (26.40)	86.15 (14.49)	86.84 (18.34)	F=0.16			
HR	84.19 (14.98)	90.45 (15.78)	75.70 (11.36)	F=13.26***	≤0.001	≤0.001	0.039
Cognition							
General cognition	-0.18 (0.91)	-0.21 (1.18)	0.66 (0.43)	F=8.36***	≤0.001	≤0.001	≤0.001
Verbal fluency	-0.22 (0.82)	-0.24 (0.80)	0.86 (0.69)	F=25.35***	≤0.001	≤0.001	≤0.001
Processing speed	-0.18 (0.45)	-0.36 (0.60)	0.01 (0.28)	F=31.56***	≤0.001	≤0.001	
Attention	-0.14 (0.77)	-0.35 (0.73)	0.60 (0.80)	F=17.28***	0.005	≤0.001	
Verbal memory	-0.18 (0.87)	-0.05 (0.74)	0.53 (0.61)	F=15.95***	≤0.001	≤0.001	
Visual memory	-0.02 (0.79)	-0.18 (0.81)	0.24 (0.67)	F=1.94***	0.048	0.002	
Visuoconstructive ability	-0.03 (0.87)	-0.14 (1.24)	0.22 (0.88)	F=2.47*		0.010	
Visuospatial perception	0.03 (1.08)	-0.26 (0.95)	0.29 (0.73)	F=7.86***			
Executive functions	-0.05 (0.69)	-0.22 (0.64)	0.39 (0.53)	F=19.72***	≤0.001	≤0.001	

Table 1. Demographic and clinical data

*$p \leq .05$; ** $p \leq .01$; ***$p \leq .001$. *Note: Cognitive domains are shown in z-scores. The ANCOVA statistic was used, with sex, age and education as covariates cognitive domains' comparisons. Fatigue (MFIS) and autonomic symptoms (COMPASS-31) are included as representative clinical measures of the core symptom domains of PCC and ME/CFS.* COMPASS: The Composite Autonomic Symptom Score; EMM: Estimated marginal means; HCs: healthy controls; ESC: electrochemical skin conductance; ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; MFIS: Modified Fatigue Impact Scale; PCC: post-COVID condition.

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