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Chronic fatigue syndrome and fibromyalgia-like symptoms are an integral component of the phenome of schizophrenia: neuro-immune and opioid system correlates.

Short title: biomarkers of fibro-fatigue symptoms in schizophrenia

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Abstract

Background: Physiosomatic symptoms are an important part of schizophrenia phenomenology. The aim of this study is to examine the biomarker, neurocognitive and symptomatic correlates of physiosomatic symptoms in schizophrenia.

Methods: We recruited 115 schizophrenia patients and 43 healthy controls and measured the Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) scale, schizophrenia symptom dimensions, and the Brief Assessment of Cognition in Schizophrenia. We measured neuro-immune markers including plasma CCL11 (eotaxin), interleukin-(IL)-6, IL-10, Dickkopf protein 1 (DKK1), high mobility group box 1 protein (HMGB1) and endogenous opioid system (EOS) markers including κ-opioid receptor (KOR), μ-opioid receptor (MOR), endomorphin-2 (EM2) and β-endorphin.

Results: Patients with an increased FF score display increased ratings of psychosis, hostility, excitement, formal though disorders, psychomotor retardation and negative symptoms as compared with patients with lower FF scores. A large part of the variance in the FF score (55.1%) is explained by the regression on digit sequencing task, token motor task, list learning, IL-10, age (all inversely) and IL-6 (positively). Neural network analysis shows that the top-6 predictors of the FF score are (in descending order): IL-6, HMGB1, education, MOR, KOR and IL-10. We found that 45.1% of the variance in a latent vector extracted from cognitive test scores, schizophrenia symptoms and the FF score was explained by HMGB-1, MOR, EM2, DKK1, and CCL11.

Conclusions: FF symptoms are an integral part of the phenome of schizophrenia. Neurotoxic immune and neurodegenerative pathways and to a lesser extent the EOS appear to drive FF symptoms in schizophrenia.

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Keywords: chronic fatigue syndrome, myalgic encephalomyelitis, schizophrenia, neuroimmunomodulation, inflammation, biomarkers.

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Introduction

Schizophrenia is a severe mental illness, which affects all essential aspects of life, such as behavioral, cognitive and psychosocial functioning (Świtaj et al., 2012). Recent findings show that the phenome of schizophrenia not only comprises symptom domains such as psychosis, hostility, excitation, mannerism (PHEM), negative symptoms, psychomotor retardation and formal thought disorders, but also physiosomatic symptoms including chronic fatigue- and fibromyalgia-like symptoms (Kanchanatawan, Thika, et al., 2018).

A relevant part of schizophrenia patients show physiosomatic symptoms reminiscent of Myalgic Encephalomyelitis (ME) / chronic fatigue syndrome (CFS) and fibromyalgia, including fatigue, gastro-intestinal (GI) and autonomic symptoms, a flu-like malaise, and muscle pain and tension (Kanchanatawan, Sriswasdi, & Maes, 2019; Waters, Naik, & Rock, 2013). Kanchanatawan et al. (2018) reported that, in schizophrenia patients, anxiety and ME/CFS-like symptoms are more important predictors of a lowered health-related quality of life (HR-Qol) than negative and PHEM symptoms (Kanchanatawan, Thika, et al., 2018). Moreover, those ME/CFS-like physiosomatic symptoms are significantly associated with negative and PHEM symptoms and with neurocognitive impairments in executive functions and episodic and memory (Waters et al., 2013). Almulla et al. (2019) found that there is a highly significant association between the physiosomatic symptoms as assessed with the Fibro-fatigue (FF) scale (Zachrisson, Regland, Jahreskog, Kron, & Gottfries, 2002) and the diagnosis of deficit schizophrenia while all 12 items of the FF scale were significantly higher in deficit schizophrenia as compared with healthy controls (Almulla, Al-Hakeim, Abed, Carvalho, & Maes, 2019). Moreover, there were significant correlations between the total FF score and PHEM and negative symptoms, psychomotor retardation and formal thought disorders (Kanchanatawan, Hemrungrojn, et al., 2018).

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There is now evidence that schizophrenia is a neuro-immune disorder (Roomruangwong et al., 2020; Smith & Maes, 1995) and that PHEM and negative symptoms, psychomotor retardation and formal thought disorders are strongly related to peripheral blood immune markers (Roomruangwong et al., 2020). The aberrations in the latter indicate activation of a) the immune-inflammatory responses system (IRS) as indicated by activated M1 macrophages with higher levels of interleukin (IL)-1 and tumor necrosis factor (TNF)-α, and Thelper (Th)-1 cells, and b) the compensatory immune-regulatory system (CIRS) as indicated by activated Th-2 cells with increased IL-4 and CCL11 or eotaxin levels, and Tregulatory (Treg) cells with increased levels of IL-10 (Roomruangwong et al., 2020).

There is also evidence that ME/CFS is associated with multiple neuro-immune aberrations including elevated levels of the pro-inflammatory cytokines IL-1β and TNF-α (Maes & Twisk, 2010; Maes, Twisk, & Ringel, 2012). A recent study reported a general upregulation of pro-inflammatory cytokine and chemokines including CCL11 (eotaxin) in ME/CFS patients as compared with controls, especially in the first phases of illness, and additionally significant associations between these immune markers and severity of illness (Montoya et al., 2017). Another study also reported increased levels of CCL2 and CCL11 in ME/CFS (Roerink et al., 2017).

Likewise, also the ME/CFS-like physiosomatic symptoms in schizophrenia are significantly associated with immune-inflammatory biomarkers. Firstly, 66.8% of the variance in a latent vector (LV) extracted from the 12 FF items could be explained by the regression on increased plasma levels of IL-1β and sIL-1RA (indicating increased IL-1 signaling), TNF-α and CCL11 (eotaxin) (Almulla et al., 2019). Moreover, the same immune-inflammatory indicants explained up to 59.4% of the variance in an integrated index of overall severity of schizophrenia

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(OSOS) conceptualized as a LV extracted from PHEM, negative symptoms, psychomotor retardation, formal thought disorders and FF symptoms as well (Almulla et al., 2019). Since this LV fits a reflective model, it may be concluded that these symptom domains are manifestations of an underlying construct, namely OSOS, which is largely predicted by neuro-immune pathways. Secondly, not only negative, PHEM and affective symptoms but also physiosomatic symptoms are associated with activation of the tryptophan catabolite (TRYCAT) pathway as assayed with IgA/IgM responses to TRYCATs (Kanchanatawan, Hemrungrojn, et al., 2018; Kanchanatawan, Sirivichayakul, Ruxrungtham, Carvalho, Geffard, Anderson, et al., 2018; Kanchanatawan, Sirivichayakul, Ruxrungtham, Carvalho, Geffard, Ormstad, et al., 2018; Kanchanatawan et al., 2017).

Recently, new biomarkers with neurotoxic activity were discovered in schizophrenia, namely increased levels of the master inflammatory protein high mobility group box 1 (HMGB1), which may damage the blood-brain-barrier (BBB) and cause neurodegenerative processes, and Dickkopf-related protein 1 **(**DKK1), a pro-inflammatory glycoprotein that functions as an antagonist of the canonical Wnt signaling pathway thereby interfering with regeneration and repair mechanisms while inducing a disassembly of synapses (Al-Dujaili, Mousa, Al-Hakeim, & Maes, 2019). A combination of HMGB1, DKK1, IL-6 and CCL11 significantly predicted PHEM and negative symptoms as well as neurocognitive impairments (Al-Dujaili et al., 2019).

We also reported that the endogenous opioid system (EOS) may contribute to the symptomatology and neurocognitive impairments in schizophrenia through increased levels of mu-opioid (MOR) and kappa-opioid (KOR) receptors, and endomorphin-2 (EM2) (Moustafa et al., 2020). There are few papers suggesting that the EOS may play a role in the pathophysiology of ME/CFS. One study showed that some patients with ME/CFS show increased opioid activity in

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their monocytes (Prieto, Subira, Castilla, Arroyo, & Serrano, 1989) while other studies suggested lowered β-endorphin levels in peripheral blood mononuclear cells (Conti et al., 1998; Panerai et al., 2002). Nevertheless, to the best of our knowledge there are no published data whether MOR, KOR and EM2 are associated with ME/CFS or with the physiosomatic symptoms of schizophrenia.

Hence, the present study aims to delineate the neuro-immune and EOS biomarkers of physiosomatic symptoms as assessed with the FF scale in schizophrenia and whether these ME/CFS-like symptoms are part of the phenome of schizophrenia.

Participants and Methods

Participants

In the present study, 115 schizophrenia patients and 43 healthy controls of both sexes, and ages between 18 and 65 years were recruited at the Psychiatry Unit at Al-Imam Al-Hussain Medical City, Karbala Governorate, Iraq during the period July 2019 until September 2019. The patients complied with the DSM-IV-TR criteria of schizophrenia. Heathy controls were family members or friends of staff members or friends of patients. Both schizophrenia patients and healthy controls were recruited from the same catchment area, namely Karbala city, Iraq. We excluded schizophrenia patients and controls who ever utilized immunomodulatory drugs such as immunosuppressive and glucocorticoids, and antioxidant supplements (therapeutic doses) three months before the study. We excluded healthy controls when they complied with the DSM-IV-TR criteria of any lifetime or current axis-1 diagnosis or showed a positive family history of any psychiatric disorder. We excluded schizophrenia patients if they suffered from: a) (auto)immune disorders including rheumatoid arthritis, diabetes mellitus, inflammatory bowel disease, and

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psoriasis; b) neuroinflammatory or neurodegenerative disorders including Parkinson's and Alzheimer's disease, multiple sclerosis, and stroke; and c) axis-1 DSM-IV-TR diagnoses other than schizophrenia including autism spectrum disorders, schizoaffective disorder, major depression, psycho-organic conditions, and bipolar disorder. Moreover, all participants showed serum C-reactive protein (CRP) levels < 6 pg/mL excluding participants with sings of overt inflammation.

All controls and patients, as well as the guardians of patients (parents or the closest family members), gave written informed consent prior to participation in our study. The study was conducted according to International and Iraq ethics and privacy laws. Approval for the study was obtained from the Institutional Review Board of the University of Karbala (418/2019) and Karbala Health Department (1331/2019), which is in compliance with the International Guidelines for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, Council for International Organizations of Medical Sciences (CIOMS) Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Measurements

Clinical assessments

A senior psychiatrist specialized in schizophrenia evaluated inclusion and exclusion criteria and made the diagnosis of schizophrenia using the Mini-International Neuropsychiatric Interview (M.I.N.I.), in a validated Arabic translation. The same day, the same senior psychiatrist assessed sociodemographic data, clinical data using a semi-structured interview, and the Fibromyalgia and Chronic Fatigue Syndrome Rating (FibroFatigue or FF) scale to measure severity of ME/CFS-like symptoms (Zachrisson et al., 2002). "The FF scale measures 12

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symptoms, namely FF1: muscle pain, FF2: muscular tension, FF3: fatigue, FF4: concentration difficulties, FF5: failing memory, FF6: irritability, FF7: sadness, FF8: sleep disturbances, FF9: autonomic disturbances, FF10: irritable bowel, FF11: headache, and FF12: a flu-like malaise. The total sum of all 12 items (FFtot) was used as an index of overall severity of fatigue and physiosomatic symptoms" (Kanchanatawan, Thika, et al., 2018). Subsequently, we dichotomized the patients into two FF subgroups according to the median FF value in the patients. We also computed a "pure" physiosomatic FF (FFsom) score (without for example neurocognitive disorders) as the sum of scores of FF1 + FF2 + FF3 + FF10 + FF11 + FF12. The same day, the same psychiatrist also measured the Scale for the Assessments of Negative Symptoms (SANS) and the negative syndrome scale of the Positive and Negative Syndrome Scale for schizophrenia (PANSS) to assess negative symptoms (Kay, Fiszbein, & Opler, 1987). We also assessed the Brief Psychiatric Rating Scale (BPRS) (Overall & Gorham, 1988) and the positive and general subscales of the PANNS in order to compute composite score reflecting PHEM symptoms, formal thought disorders (FTD) and psycho-motor retardation (PMR) (Almulla et al., 2019).

On the same day, a well-trained psychologist who was blinded to the clinical diagnosis, completed the Brief Assessment of Cognition in schizophrenia (BACS) (Keefe et al., 2004). Episodic memory was probed using the List Learning test; working memory with the Digit Sequencing Task; semantic memory and verbal fluency with the Category Instances and Controlled Word Association tests; attention with the Symbol Coding test; and executive functions with the Tower of London. Tobacco Use Disorder (TUD) was diagnosed using DSM-IV-TR criteria. The Body mass index (BMI) was assessed on the same day as the clinical interview as body weight in kg / length in m^2.

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Assays

Fasting (overnight fast) venous blood was sampled between 8.00 and 9.00 a.m. (5 mL) utilizing disposable needle and plastic syringes; the samples were transferred into a clean plain tube and blood was left at room temperature for 15 min for clotting, centrifuged 3000 rpm for 10 min, and then serum was separated and transported into two Eppendorf tubes to be stored at -80 °C until thawed for assay. We employed commercial ELISA sandwich kits to assay serum DKK1, CCL11, IL-10 and HMGB1 (Elabscience®, Inc. CA, USA) and IL-6 (Melsin Medical Co, Jilin, China). The CCL11 (sensitivity=9.38 pg/mL), DKK1 (sensitivity=18.75 pg/mL), HMGB1 (sensitivity=18.75 pg/mL), and IL-6 (sensitivity=0.1 pg/mL) concentrations all exceeded the sensitivity of the assays. The intra-assay coefficient of variation (CV) (precision within an assay) for all assays was were < 10.0%. Serum CRP was measured using a kit supplied by Spinreact®, Spain. The test is based on the principle of latex agglutination.

Statistical analysis

Analysis of variance (ANOVA) was used to check differences in scale variables between groups and analysis of contingency tables (χ^2-test) to assess associations among categorical variables. In order to assess the associations between biomarkers, and clinical and cognitive test scores, we used correlation matrices based on Pearson's product-moment or Spearman's rank-order correlation coefficients. Univariate and multivariate general linear model (GLM) analysis was employed to check the associations between biomarkers and diagnostic groups (controls versus schizophrenia dichotomized into two groups, namely high versus low FF values) while adjusting for possible intervening variables such as sex, age, TUD, BMI, and education. Tests for between-subject effects were performed to ascertain the associations between diagnostic classes

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and biomarkers while effect sizes were estimated employing partial eta-squared values. GLM-generated estimated marginal mean (SE) values were calculated and we conducted protected pairwise comparisons among treatment means. Multiple regression analysis was used to delineate the significant biomarkers predicting FFtot and FFsom scores using biomarkers, symptom domains, and cognitive test scores as explanatory variables in an automatic stepwise method with a p-to-enter of 0.05 and p-to-remove 0.06. All results were checked for R^2 change and multicollinearity using VIF and tolerance values. Statistical tests were 2-tailed and a p-value of 0.05 was used for statistical significance. All statistical analyses were performed using IBM SPSS windows version 25, 2017.

Partial least squares (LSD) analysis (Ringle, 2015) was employed to delineate a) the associations between the biomarkers entered as input variables and neurocognitive and symptom domains scores entered as output variables; and b) whether the total FF score belongs to the same latent vector as all other symptom profiles. Data were entered as single indicators (the biomarkers) or as a latent vectors extracted from symptoms dimensions (including the FF score) and the neurocognitive test scores (Al-Hakeim, Almulla, & Maes, 2019). PLS path analysis was performed using 5000 bootstrap samples only when: a) the overall quality of the model as indicated by Standardized Root Mean Squared Error (SRMR) < 0.080 was adequate; b) the latent vectors extracted from indicators (symptoms and cognitive test results) had adequate reliability as indicated by and average variance extracted (AVE) > 0.500, Cronbach alpha > 0.7, composite reliability > 0.7, rho_A > 0.80; c) all indicators loaded highly (>0.400 and by preference > 0.660) at p<0.001 on the latent vector; and d) construct cross-validated redundancies are adequate (Ringle, 2015). We employed complete bootstrapping (5000 subsamples) and PLS path modeling to compute path coefficients with p-values (Luo, He, Zhang, Ou, & Fan, 2019).

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We employed multilayer perceptron Neural Network (NN) models to examine the more complex relationships between biomarkers and relevant background variables entered as input variables predicting the FFtot score entered as outcome variable. We used an automated feedforward architecture model to train the network with two hidden layers with up to 8 nodes and employed minibatch training with gradient descent and 250 epochs. Stopping rule was one consecutive step with no further decrease in the error term. The study group was split in three sets, namely a training set to estimate the network parameters (46.67% of all participants), a testing set to prevent overtraining (20.0%) and a holdout set to evaluate the final network (33.33%). Error, relative error, and importance and relative importance of all input variables were computed.

Results

Socio-demographic data

The sociodemographic data of the participants are shown in **Table 1.** There were no significant differences in age, sex ratio, urban/rural residence ratio, BMI and TUD between the three study groups. There were somewhat more schizophrenia subjects with high FF scores who were single as compared with controls. Significantly more schizophrenia participants were unemployed as compared with healthy controls. Education was somewhat lower in schizophrenia patients with high FF scores as compared with the two other groups. There were no differences in age at onset between schizophrenia patients with and without increased FF scores. Table 1 also shows the FFtot scores are significantly higher in schizophrenia patients than in controls.

Associations between diagnosis and biomarkers

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Table 2 shows a significant association between diagnostic classes and biomarkers with an effect size of 0.265 while there were no significant effects of sex, age, education, BMI and TUD. Tests for between-subject effects and **Table 3** (with GLM-generated estimated marginal mean values and results of protected pairwise comparisons among groups) indicates that IL-6, DKK1, HMGB1, EM2, KOR and MOR were significantly higher in schizophrenia than in healthy controls, while IL-10 was significantly higher in schizophrenia patients with lower FF scores than in controls. The top-4 important variables (evaluated using effect sizes) were in descending order: HMGB1, KOR and EM2 and MOR. These intra-class differences remained significant after p-correction.

Multivariate GLM analysis showed a significant association between the three study classes and neurocognitive probe results ($F=89.71$, $df=14/290$, $p<0.001$). All confounding variables were non-significant, except years of education ($F=2.67$, $df=7/145$, $p=0.012$, partial $\eta^2=0.114$). Tests for between-subjects' effects and **Electronic Supplementary File (ESF), Table 1** shows that all cognitive test results (except Category Instances) were significantly different between the three classes and decreased from controls → schizophrenia with low FF scores → schizophrenia with higher FF scores. Category Instances scores were significantly lower in schizophrenia than in controls.

There was a significant association between diagnostic classes and symptoms domains ($F=28.27$, $df=16/290$, $p<0.001$, partial $\eta^2=0.609$) and ESF, table 1 shows that all symptom domain scores are strongly associated with the diagnostic classes and that all symptom domains are significantly different between the diagnostic groups and increase from controls to schizophrenia with low FF scores to schizophrenia with higher FF scores while mannerism was significantly higher in schizophrenia than in controls.

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Effects of background variables

As described above, there were no significant effects of age, sex, BMI and TUD on the biomarkers. In patients with schizophrenia, we examined the effects of antipsychotic drug administration on the FFtot score by means of univariate GLM analysis and found no significant effects of clozapine ($F=3.10$, $df=1/145$, $p=0.08$), haloperidol ($F=0.02$, $df=1/145$, $p=0.888$), risperidone ($F=1.67$, $df=1/145$, $p=0.20$), and quetiapine ($F=0.46$, $df=1/145$, $p=0.50$). Previously, we reported that (in the same study sample) there are no significant effects of these drugs on the symptom domains and cognitive tests as well (Al-Dujaili et al., 2019).

Clinical, cognitive and biomarker correlates of the FFtot and FFsom scores

Inspection of intercorrelation matrices showed that in the total study group there were significant associations between the FFtot score and PHEM symptoms, the PANSS negative subscale score, psychomotor retardation and formal thought disorders (all $r>0.500$, $p<0.001$, $n=158$). In the restricted study group of schizophrenia patients, the FFtot score was associated with psychosis ($r=0.400$, $p<0.001$, all $n=115$), hostility ($r=0.415$, $p<0.001$), excitation ($r=0.403$, $p<0.001$), mannerism ($r=0.262$, $p=0.005$), PANSS negative score ($r=0.330$, $p<0.001$), psychomotor retardation ($r=0.433$, $p<0.001$), and formal thought disorders ($r=0.373$, $p<0.001$). The FF score was significantly associated with IL-6 ($r=0.291$, $p<0.001$, all $n=158$), HMGB1 ($r=0.249$, $p=0.002$), EM2 ($r=0.223$, $p=0.005$), KOR ($r=0.219$, $p=0.006$) and MOR ($r=0.242$, $p=0.002$). In the total study group, there were also significant correlations between the FF score and the different cognitive test scores (all $r>-0.398$, $p<0.001$, $n=158$). These associations remained significant in the restricted study sample of schizophrenia patients: list learning ($r=-0.454$,

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p<0.001, all n=115), digit sequencing task (r=-0.526, p<0.001), token motor task (r=-0.365, p<0.001), category instances (r=-0.202, p=0.031), COWA (r=-0.448, p<0.001), symbol coding (r=-0.293, p=0.001) and Tower of London (r=-0.372, p<0.001).

Table 4 shows the results of different stepwise multiple regression analyses with the FFtot or FFsom scores as dependent variables and biomarkers, cognitive test scores, and symptom domains as independent variables while allowing for the effects of age, sex, and education. Regression #1 shows that 43.7% of the variance in the FF score was explained by the regression on Token Motor Task, Digit Sequencing Task, and List Learning (all inversely associated). **Figure 1** shows the partial regression of the FFtot score on the Digit Sequencing Task probe scores. Regression #2 shows that 55.1% of the variance in the FFtot score can be explained by the regression on digit sequencing task, Tower of London, IL-10, token motor task, and age (all inversely associated) and IL-6 (positively associated). Regression #3 shows that 22.3% of the variance in the FF score was explained by IL-6 and HMGB1 (both positively associated) and IL-10 and education (both inversely associated). **Figure 2** shows the partial regression of FFtot on HMGB1. Regression #4 shows that a considerable part of the variance in FFtot (42.4%) was explained by the regression on psychosis and IL-6 (both positively) and IL-10 and β-endorphin (both inversely). Table 4 shows the results of regression analyses with the FFsom score as dependent variable. We found that hostility (positively) and education and IL-10 (inversely) explained 30.7% of the variance in the FFsom score (see regression #5). **Figure 3** shows the regression of FFsom on hostility scores. Regression #6 shows that 12.9% of the variance in FFsom was explained by the combined effects of EM2 and IL-6 (both positively) and education (inversely associated). Regression #7 shows that 39.9% of the variance in FFsom was explained by the

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combined effects of IL-10, token motor task, digit sequencing task, Tower of London and age (all negatively associated).

Results of PLS analysis

Figure 4 shows the results of PLS path analysis with the biomarkers as input variables and symptom domains (including FFtot scores) and cognitive tests results as output variables. The model shows an accurate fit with SRMR=0.029 and the latent vector extracted from the symptom's domains and cognitive tests (denoted as OSOS) had adequate reliability with AVE=0.741, Cronbach alpha=0.975, rho_A=0.984 and composite reliability=0.978. All indicators showed loadings > 0.680 (at $p<0.001$) on this OSOS LV, except Token Motor Task (loading=0.414, $p<0.001$), which was however sufficient to be included. The FFtot score showed a loading of 0.681 ($p<0.001$). Confirmatory Tetrad Analysis shows that the latent vector OSOS fitted a reflective model whilst blindfolding showed a good cross-validated redundancy of 0.328. We found that 45.2% of the variance in this latent vector was explained by the regression on HMGB1, MOR, EM2, DKK1 and CCL11. The other biomarkers were non-significant and, therefore, deleted from the model.

Results of neural networks

The final neural network was trained with 12 units, 2 hidden layers namely five in hidden layer 1 and four in hidden layer 2 while hyperbolic tangent was used as activation function in the hidden layers and identity in the output layer and sum of squares was used as the error term. The sum of squares in the testing set (4.956) was much lower than that in the training set (31.107) while also the relative error was lower in the testing than in the training set (0.644 versus 0.798,

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respectively) indicating the neural network model learned to generalize from the trend. The relative error in the holdout set was 0.889 and Spearman's rank order coefficient between the predicted FFtot value and the actual FFtot value was r=0.502, p,0.001, n=158). **Figure 5** shows the importance and relative importance of the input variables. IL-6, HMGB1, and education were the top-3 most important determinants of the predictive power of the model, and MOR, KOR and IL-10 followed at a distance.

Discussion

FF symptoms are an integral part of schizophrenia

The first major findings of this study are that schizophrenia patients show significantly increased FF scores as compared with controls and that around 50% of the patients show very high FF scores. These findings agree with those of a previous study showing that more than 50% of schizophrenia patients suffer from physiosomatic symptoms as assessed with the FF scale (Kanchanatawan et al., 2017). Moreover, another study showed that patients with deficit schizophrenia show highly significantly increased FF scores as compared with normal controls (Almulla et al., 2019). As such, a meaningful subset of schizophrenia subjects suffer from ME/CFS-like symptoms including increased levels of chronic fatigue, sleep disorders, a flu-like malaise, gastro-intestinal and autonomic symptoms, and fibromyalgia-like symptoms including muscle pain and muscle tension, and neurocognitive impairments (Kanchanatawan et al., 2019; Kanchanatawan, Sriswasdi, et al., 2018). These findings extend the results of previous studies reporting increased physiosomatic symptoms in schizophrenia including: a) elevated fatigue and lowered energy, sleep and gastro-intestinal symptoms (Chen, 2017; Hedlund, Gyllensten, & Hansson, 2015; Palmese et al., 2011; Skapinakis, Lewis, & Meltzer, 2000) and b) fibromyalgia-

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like symptoms including hyperalgesia and change in pain perception, (Zachrisson et al., 2002). Previously, we reported that subjective cognitive complaints (SCCs), as measured with the FF score, are associated with test scores on neurocognitive probes (Sirivichayakul, Kanchanatawan, Thika, Carvalho, & Maes, 2019). Not only schizophrenia, but also ME/CFS is accompanied by cognitive impairments in information processing speed, memory, and working memory (Cockshell & Mathias, 2010; Deluca et al., 2004; Jason et al., 2002; Lange et al., 2005).

The second major finding of this study is that an increased FFtot score is associated with a) symptoms domains of schizophrenia phenomenology including negative symptoms, psychosis, excitation, and formal thought disorders; b) psychomotor retardation and scores on the token motor task (inversely), and c) lowered scores on probes assessing episodic and semantic memory, executive functions and working memory. These results extend those of previous reports showing that FF symptoms are significantly associated with negative and PHEM symptoms (Almulla, Al-Hakeim, & Maes, 2020; Kanchanatawan, Thika, et al., 2018) and additionally with psychomotor retardation and formal thought disorders (Almulla et al., 2020). It is interesting to note that memory disorders may increase risk to develop false memories and, consequently, psychosis (Corlett et al., 2007; Harvey, Patterson, Potter, Zhong, & Brecher, 2006; Tamminga, Buchanan, & Gold, 1998) and that impaired executive functions may mediate fatigue (Dobbs, Dobbs, & Kiss, 2001).

Nevertheless, the current study found that a latent vector extracted from all cognitive tests and symptom domains, including the FFtot score, loaded highly on all those indicators and additionally showed adequate internal consistency and predictive validity while fitting a reflective model. These results agree with a previous study, which reported that all these schizophrenia features are reflective manifestations of a common underlying construct, namely OSOS, which is the common cause of all its manifestations (Almulla et al., 2020).

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Immune and EOS biomarkers of physiosomatic symptoms in schizophrenia

The results of the present study show that a combination of pro-inflammatory and neurotoxic signals (IL-6, HMGB1) and lowered Treg activity (IL-10) is associated with the FF score. These results extent those of previous reports that the FF score is strongly predicted by a) indicants of IRS/CIRS activation, namely TNF-α, IL-1β, sIL-1RA and sTNFR1 and CCL11 (Almulla et al., 2020), and b) indicants of TRYCAT pathway activation, namely increased IgA responses to neurotoxic TRYCATS xanthurenic acid, picolinic acid and 3-OH-kynurenine (Kanchanatawan, Sirivichayakul, Ruxrungtham, Carvalho, Geffard, Ormstad, et al., 2018). Although Almulla et al. (2020) detected that CCL1 significantly predicts the FF score no such findings were reported in the present study (Almulla et al., 2020). Differences in study samples may explain these discrepant results with higher CCL11 levels in the groups of deficit schizophrenia (Almulla et al., 2020) and no differences in the current group which comprises relatively few patients with deficit schizophrenia. All in all, the results of our studies in Thai and Iraq patients indicate that the severity of FF symptoms is predicted by IRS (TNF-α, IL-1β, sIL-1RA, sTNFR1, CCL11, HMGB1, DKK1, IgA directed against TRYCATs) and CIRS (sIL-1RA, sTNFR1, IL-10) biomarkers. This is a first study reporting that increased EOS biomarkers are associated with ME/CFS-like symptoms in schizophrenia, namely MOR, KOR and EM2 (positively) and β-endorphin (inversely). As described in the Introduction, there are only few studies in ME/CFS and these reported contradictory results (Conti et al., 1998; Panerai et al., 2002; Prieto et al., 1989).

The results of the current study show that a large part of the variance (45.1%) in the OSOS index, including FF symptoms and neurocognitive impairments is explained by a combination of

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neuro-immune and EOS biomarkers, in descending order of relevance: HMGB1, MOR, EM2, DKK1 and CCL11. These data extend the findings of Almulla et al. (2020) who reported that pro-inflammatory signals (IL-1β, sIL-1 receptor antagonist, TNF-α, CCL11) explained 59.4% of the variance in PHEM and negative symptoms combined with the FF score (Almulla et al., 2020), affective scores, psychomotor retardation and formal thought disorders. This is important as the common latent trait underpinning those late phenome manifestations of schizophrenia is predicted by the combined effects of neuro-immune and EOS biomarkers.

Mechanistic explanations

Recently, we have discussed that IRS (e.g. IL-1β, TNF-α, IL-6, IFN-γ, CCL11, TRYCATs) as well as CIRS (e.g. IL-4) products may induce neurotoxic and excitotoxic effects and, therefore, may induce impairments in episodic, semantic and working memory, executive functions and formal thought disorders and, consequently, schizophrenia symptom domains including PHEM and negative symptoms as well as psychomotor retardation (Maes et al., 2020; Sirivichayakul et al., 2019). The same immune products are also associated with ME/CFS-like symptoms (Gerwyn Morris, F Carvalho, Anderson, Galecki, & Maes, 2016) and fibromyalgia. (Andrés-Rodríguez et al., 2019) ME/CFS (which includes fibromyalgia symptoms) is conceptualized as a neuro-inflammatory disorder characterized by diverse immune and autoimmune aberrations while immune triggers are often associated with the onset or maintenance of the disease (Maes, 2011; Maes, Bosmans, & Kubera, 2015; Maes et al., 2013; Maes, Twisk, & Johnson, 2012; G. Morris & Maes, 2013). Thus, activation of IRS/CIRS coupled with lowered immune-regulation may underpin ME/CFS-like and the other symptom domains of schizophrenia. Moreover, HMGB1 is a biomarker of neuro-immune activation which stimulates the production of IL-6 and TNF-α (Al-

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Dujaili et al., 2019; Kwak et al., 2015), and may cause breakdown of the BBB as well as neurodegeneration. (Festoff, Sajja, van Dreden, & Cucullo, 2016). Second, DKK1 is a pro-inflammatory glycoprotein (Chae & Bothwell, 2019) that may cause breakdown of the BBB, interfere with neurogenesis, repair and tissue regeneration and induce neuroprogression (Chae & Bothwell, 2019; Gerwyn Morris, Berk, Galecki, Walder, & Maes, 2016; Orellana et al., 2014; Salinas, 2013; Scali et al., 2006).

Recently, we reviewed that the EOS may contribute to schizophrenia symptomatology and neurocognitive impairments (Moustafa et al., 2020). For example, KOR activators or agonists have psychotomimic properties and may induce hallucinations and negative-like symptoms, neurocognitive deficits and psychomotor retardation (Clark & Abi-Dargham, 2019; Land et al., 2008; Nemeth et al., 2010; Shekhar, 2019). KORs are also implicated in chronic inflammatory and visceral pain, fatigue, weakness, and autonomic symptoms (Mysels, 2009). EM2 may trigger excitation, a bell-shaped curve for locomotor enhancement and place aversion, and may stimulate postsynaptic MOR causing hyperpolarization of excitatory interneurons (Y.-B. Chen et al., 2015; Heinke, Gingl, & Sandkühler, 2011). On the other hand, β-endorphin and MOR have antinoceptive effects and may exert CIRS activities by suppressing the primary immune response in schizophrenia (Moustafa et al., 2020). Future research should examine the EOS in ME/CFS and fibromyalgia to delineate the role of those opioids.

Limitations

The results of our study should be interpreted with regard to its limitations. First, this is a case-control study and, therefore, no causal inferences can be firmly drawn. Second, it would have been more interesting if we had measured more IRS and CIRS biomarkers as well as levels of dynorphin and enkephalins. Future research should investigate whether the onset of physiosomatic

symptoms in ME/CFS and schizophrenia may have another shared etiopathology including leaky gut with breakdown of the gut tight and adherens junctions (Maes, Mihaylova, & Leunis, 2007; Maes, Sirivichayakul, Kanchanatawan, & Vodjani, 2019; Slyepchenko et al., 2017).

Conclusions

In schizophrenia, there is an association between ME/CFS-like symptoms and negative symptoms, psychosis, hostility, excitation, formal though disorders, and psychomotor retardation as well as impairments in episodic, semantic and working memory, attention and executive functions. ME/CFS-like symptom are an integral part of the phenome of schizophrenia. A large part of the variance in the FF score is positively associated with neurotoxic immune and neurodegenerative markers including IL-6, HMGB1, DKK1, and EOS biomarkers, including MOR, KOR, END2. The FF score is inversely associated with IL-10 and β-endorphin levels. Neurotoxic immune and neurodegenerative pathways and lowered immune-regulation and alterations in the EOS appear to drive FF symptoms in schizophrenia.

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Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

Author's contributions

All the contributing authors have participated in preparation of the manuscript.

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Table 1. Demographic and biomarker data in healthy controls (HC), and schizophrenia (SCZ) patients divided into those with low versus high scores on the Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue (FF) scale

Variables	HC[A] n=43	SCZ+FF < median [B] n=55	SCZ+FF ≥ median [C] n=60	F/Ψ/χ^2	df	p
Age (years)	33.2 (11.1)	37.2 (9.7)	35.3 (12.4)	1.71	2/155	0.184
Sex (Female/Male)	19/24	15/40	22/38	3.08	2	0.214
Single/married	12/31 [C]	25/30	32/28 [A]	6.69	2	0.035
Residency Urban/Rural	30/13	36/19	39/21	0.29	2	0.864
BMI (kg/m^2)	27.9 (4.1)	29.0 (4.6)	28.9 (4.7)	0.94	2/155	0.394
Education (years)	11.1 (3.6) [C]	11.2 (4.5) [C]	8.4 (4.5) [A,B]	7.64	2/155	0.001
Age of onset of schizophrenia (years)	-	28.6 (8.7)	28.3 (9.5)	0.02	1/113	0.888
Employment (No/Yes)	17/26 [B,C]	36/19 [A]	43/17 [A]	11.63	2	0.003
FFtot score	8.4 (3.9) [B,C]	11.5 (4.6) [A,C]	29.9 (8.8) [A,B]	179.89	2/155	<0.001
TUD (No/Yes)	30/13	44/11	40/20	2.71	2	0.258
Interleukin-6 (pg/mL)*	4.8 (5.7) [C]	5.6 (5.3)	7.4 (6.0) [A]	7.42	2/155	0.001
Interleukin-10 (pg/mL)	11.0 (3.8) [B, C]	14.2 (7.2) [A]	13.0 (4.8) [A]	3.89	2/155	0.022
CCL11 (pg/mL)	178.3 (46.8) [B,C]	209.7 (66.2) [A]	205.5 (67.8) [A]	2.43	2/155	0.091
DKK1 (pg/mL)*	697.5 (525.7) [B,C]	924.9 (546.0)[A]	1010.4 (646.2) [A]	4.86	2/155	0.009
HMGB1 (ng/mL)*	7.9 (7.9) [B,C]	21.2 (12.2) [A]	20.60 (10.6) [A]	37.45	2/155	<0.001
β-endorphin (pg/mL)*	20.6 (14.6)	22.7 (21.7)	19.5 (10.1)	0.22	2/155	0.803
Endomorphin-2 (pg/mL)*	260.7 (226.5) [B,C]	398.3 (292.9) [A]	420.3 (259.1) [A]	9.20	2/155	<0.001
KOR (ng/mL)*	4.04 (3.40) [B,C]	7.00 (8.16) [A]	7.99 (7.44) [A]	14.37	2/155	<0.001
MOR (pg/mL)	3.00 (2.13) [C]	3.80 (2.27)	4.52 (2.73) [A]	8.32	2/155	<0.001

All results are shown as mean (SD); [A,B,C]: pairwise comparisons between group means; *: Processed in Ln transformation.
BMI: Body mass index, CCL11: CC-motif chemokine 11 or eotaxin; DKK1: Dickkopf protein 1, HMGB1: high mobility group box 1 protein, KOR: κ-opioid receptor, MOR: μ-opioid receptor, and TUD: tobacco use disorder.

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Table 2: Differences in biomarkers between healthy controls (HC), and schizophrenia (SCZ) patients divided into those with low versus high scores on the Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue (FF) scale.

Tests	Dependent variables	Explanatory variables	F	df	p	Partial η^2
Multivariate	All 9 Biomarkers	HC and 2 FF groups	5.69	18/284	<0.001	0.265
		Sex	1.31	9/142	0.235	0.077
		Age	0.74	9/142	0.676	0.045
		Education	0.92	9/142	0.513	0.055
		BMI	0.74	9/142	0.672	0.045
		TUD	0.38	9/142	0.942	0.024
Between-subject effects	IL-6	HC and 2 FF groups	6.89	2/150	0.001	0.084
	IL-10		3.05	2/150	0.050	0.039
	CCL11		2.90	2/150	0.058	0.037
	DKK1		5.05	2/150	0.008	0.063
	HMGB1		33.05	2/150	<0.001	0.306
	β-EP		0.64	2/150	0.529	0.008
	EM2		9.07	2/150	<0.001	0.108
	KOR		13.55	2/150	<0.001	0.153
	MOR		9.02	2/150	<0.001	0.107

All results of multivariate GLM analysis with the biomarkers as dependent variables and diagnosis as explanatory variable while adjusting for extraneous variables.
BMI: Body mass index, βEP: β-endorphin, CCL11: CC-motif chemokine 11 or eotaxin; DKK1: Dickkopf protein 1, EM2: Endomorphin-2, HMGB1: high mobility group box 1 protein, IL: interleukin, KOR: κ-opioid receptor, MOR: μ-opioid receptor, and TUD: tobacco use disorder.

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Table 3: Model-generated (see Table 2) estimated marginal mean values in healthy controls (HC), and schizophrenia patients divided into those with low versus high scores on the Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue (FF) scale

Dependent Variables (in z scores	**HC [A] n=43**	**SCZ+FF < median [B] n=55**	**SCZ+FF ≥ median [C] n=60**
IL-6	-0.366 (0.150) [B,C]	0.087 (0.134) [A]	0.371 (0.138) [A]
IL-10	-0.332 (0.157) [B]	0.164 (0.141) [A]	0.018 (0.145)
CCL11	-0.334 (0.158)	0.127 (0.142)	0.080 (0.146)
DKK1	-0.383 (0.156) [B,C]	0.194 (0.141) [A]	0.191 (0.144) [A]
HMGB1	-0.898 (0.133) [B,C]	0.340 (0.119) [A]	0.362 (0.122) [A]
β-EP	0.023 (0.160)	0.137 (0.144)	-0.086 (0.147)
EM2	-0.497 (0.152) [B.C]	0.149 (0.137) [A]	0.333 (0.141) [A]
KOR	-0.588 (0.147) [B,C]	0.143 (0.132) [A]	0.403 (0.135) [A]
MOR	-0.47 1 (0.152) [B,C]	0.071 (0.137) [A]	0.386 (0.140) [A]

[A,B,C]: pairwise comparisons between group means.

βEP: β-endorphin, CCL11: CC-motif chemokine 11 or eotaxin; DKK1: Dickkopf protein 1, EM2: Endomorphin-2, HMGB1: high mobility group box 1 protein, IL: interleukin, KOR: κ-opioid receptor, MOR: μ-opioid receptor

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Table 4 Results of multiple regression analysis with the total Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue scale total score (FFtot) or the FF somatic subdomain (FFsom) as dependent variables

Regression	Explanatory variables	β	t	p	F_{model}	df	p	R^2
#1. FFtot	**Model**				**39.54**	**3/153**	**<0.001**	**0.437**
	Digit sequencing task	-0.405	-4.96	<0.001				
	Token motor task	-0.175	-2.63	0.009				
	List learning	-0.217	-2.63	0.009				
#2. FFtot	**Model**				**30.26**	**6/148**	**<0.001**	**0.551**
	Digit squencing task	-0.353	-3.70	<0.001				
	Tower of London	-0.327	-3.47	0.001				
	IL-10	-0.161	-2.83	0.005				
	Token motor task	-0.151	-2.55	0.012				
	Age	-0.137	-2.41	0.017				
	IL-6	0.129	2.19	0.030				
#3. FFtot	**Model**				**10.83**	**4/151**	**<0.001**	**0.223**
	IL-6	0.251	3.33	0.001				
	HMGB1	0.321	3.89	<0.001				
	IL-10	-0.202	-2.50	0.013				
	Education	-0.168	-2.30	0.023				
#4. FFtot	**Model**				**27.76**	**4/151**	**<0.001**	**0.424**
	Psychosis	0.598	8.89	<0.001				
	IL-10	-0.146	-2.29	0.023				
	IL-6	0.169	2.52	0.013				
	β-Endorphin	-0.133	-2.10	0.037				
#5. FFsom	**Model**				**22.31**	**3/151**	**<0.001**	**0.307**
	Hostility	0.526	7.44	<0.001				
	IL-10	-0.173	-2.49	0.014				
	Education	-0.138	-2.00	0.048				
#6. FFsom	**Model**				**7.43**	**3/151**	**<0.001**	**0.129**
	IL-6	0.201	2.53	0.013				

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	Education	-0.180	-2.33	0.021				
	Endomorphin 2	0.157	2.00	0.048				
#7. FFsom	**Model**				**19.69**	**5/148**	**<0.001**	**0.399**
	Digit Sequencing Task	-0.246	-2.25	0.026				
	Token Motor Task	-0.190	-2.78	0.006				
	Tower of London	-0.340	-3.13	0.002				
	IL-10	-0.169	-2.59	0.011				
	Age	-0.155	-2.35	0.020				

IL: interleukin; HMGB: high mobility group box 1 protein

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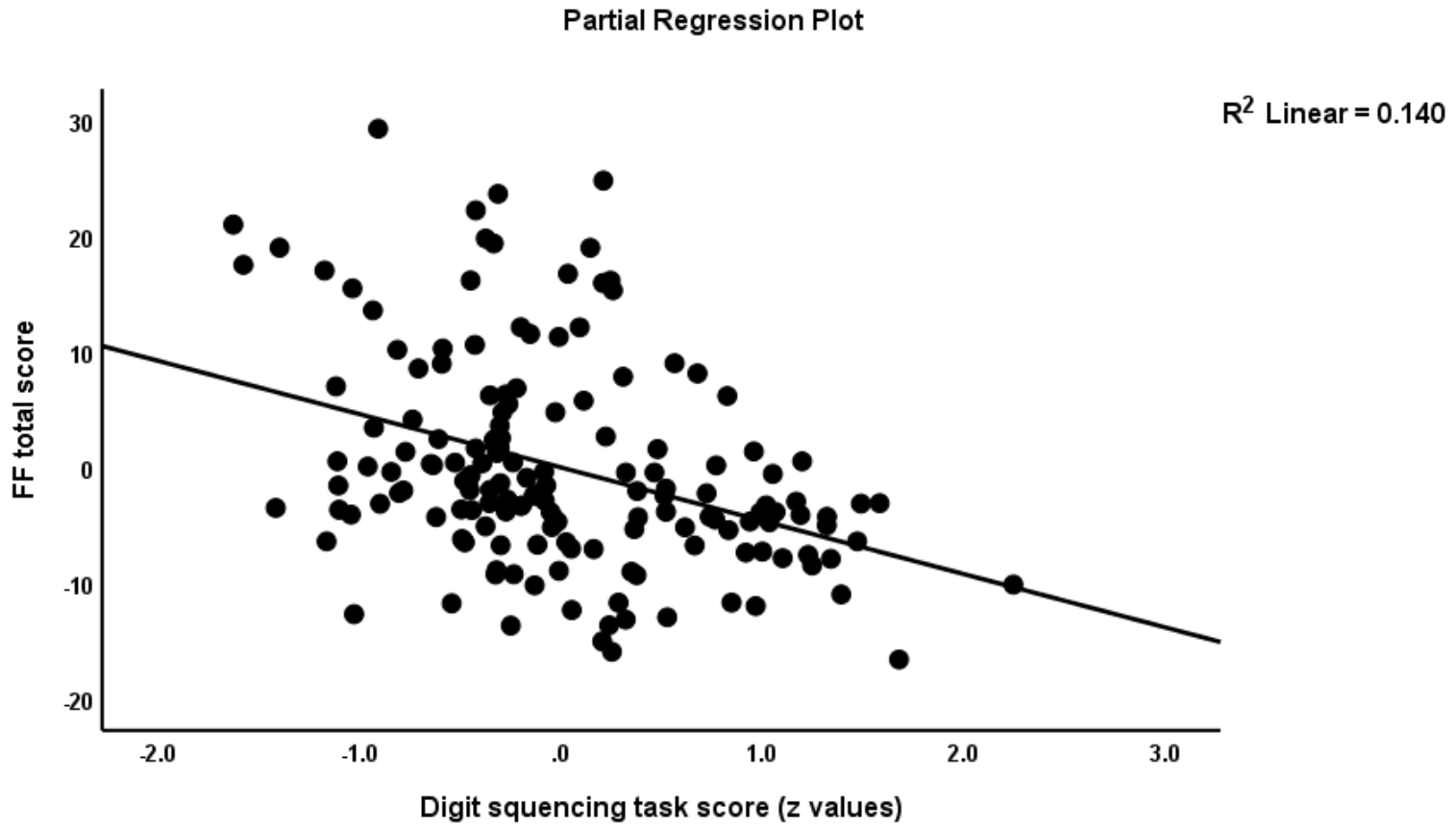

Figure 1 Partial regression of the Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue (FFtot) score on the Digit Sequencing Task probe scores.

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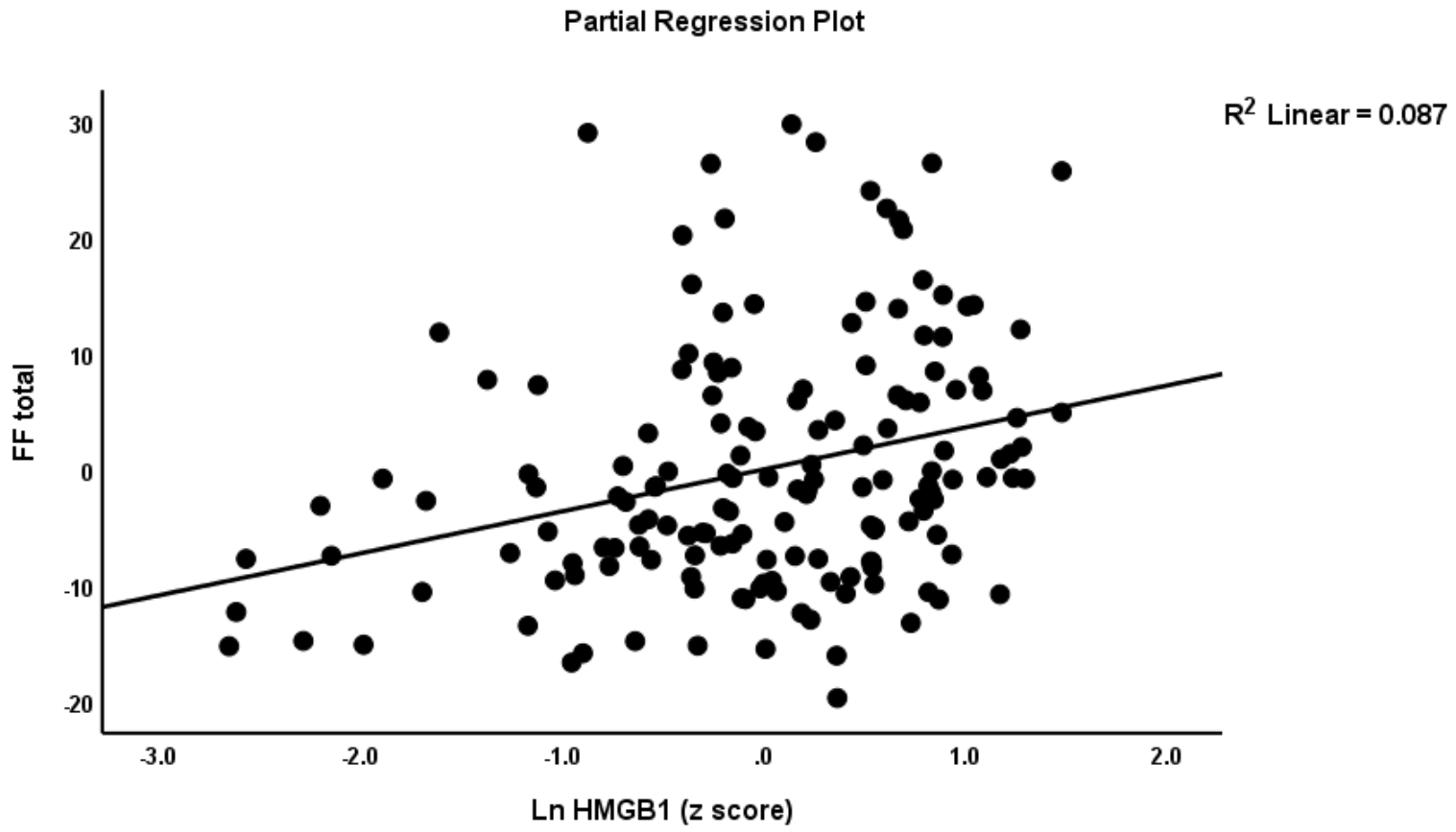

Figure 2 Partial regression of the Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue (FFtot) score on high mobility group box 1 protein (HMGB1).

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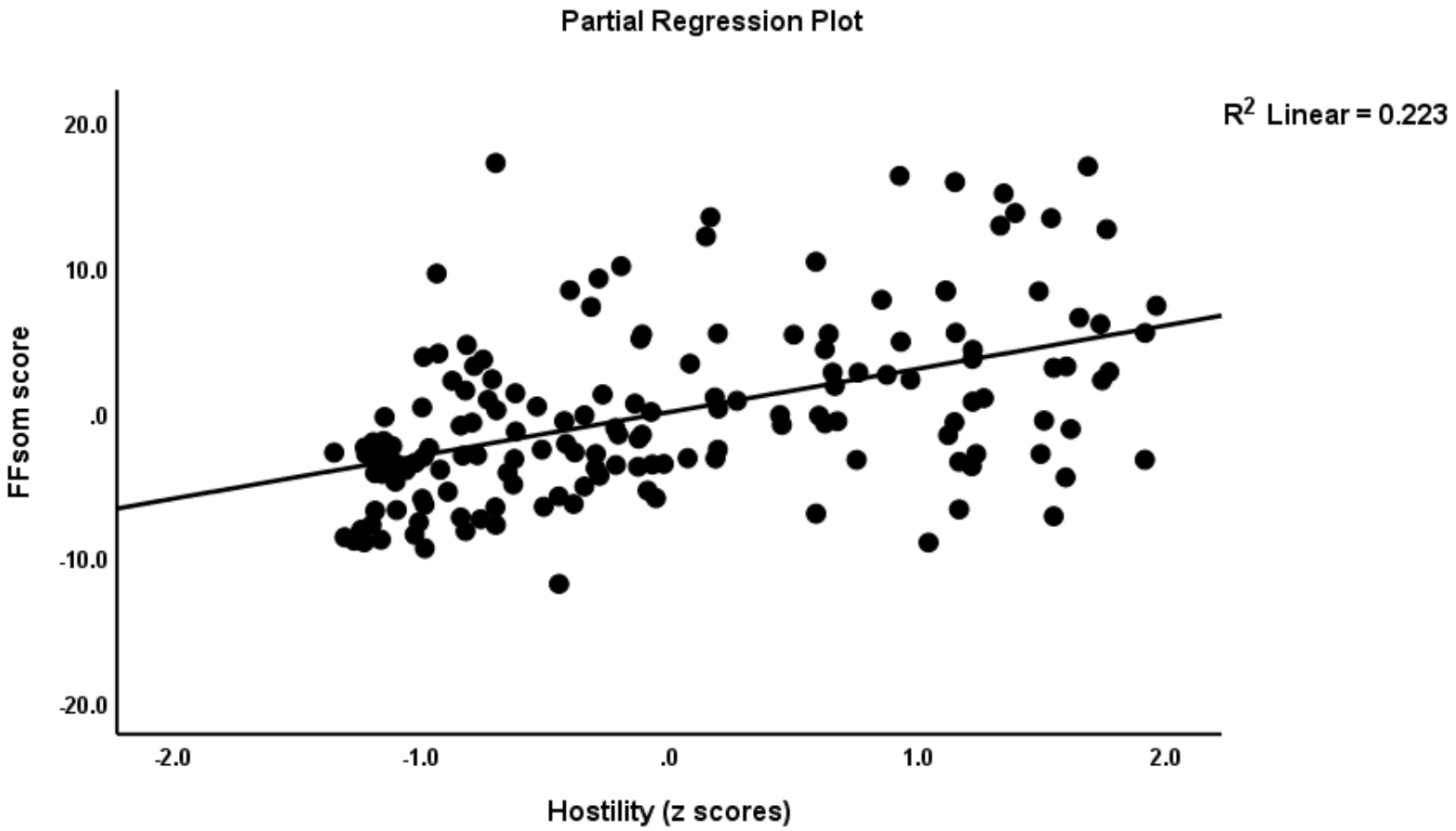

Figure 3 Partial regression of the Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue (FFtot) score on hostility

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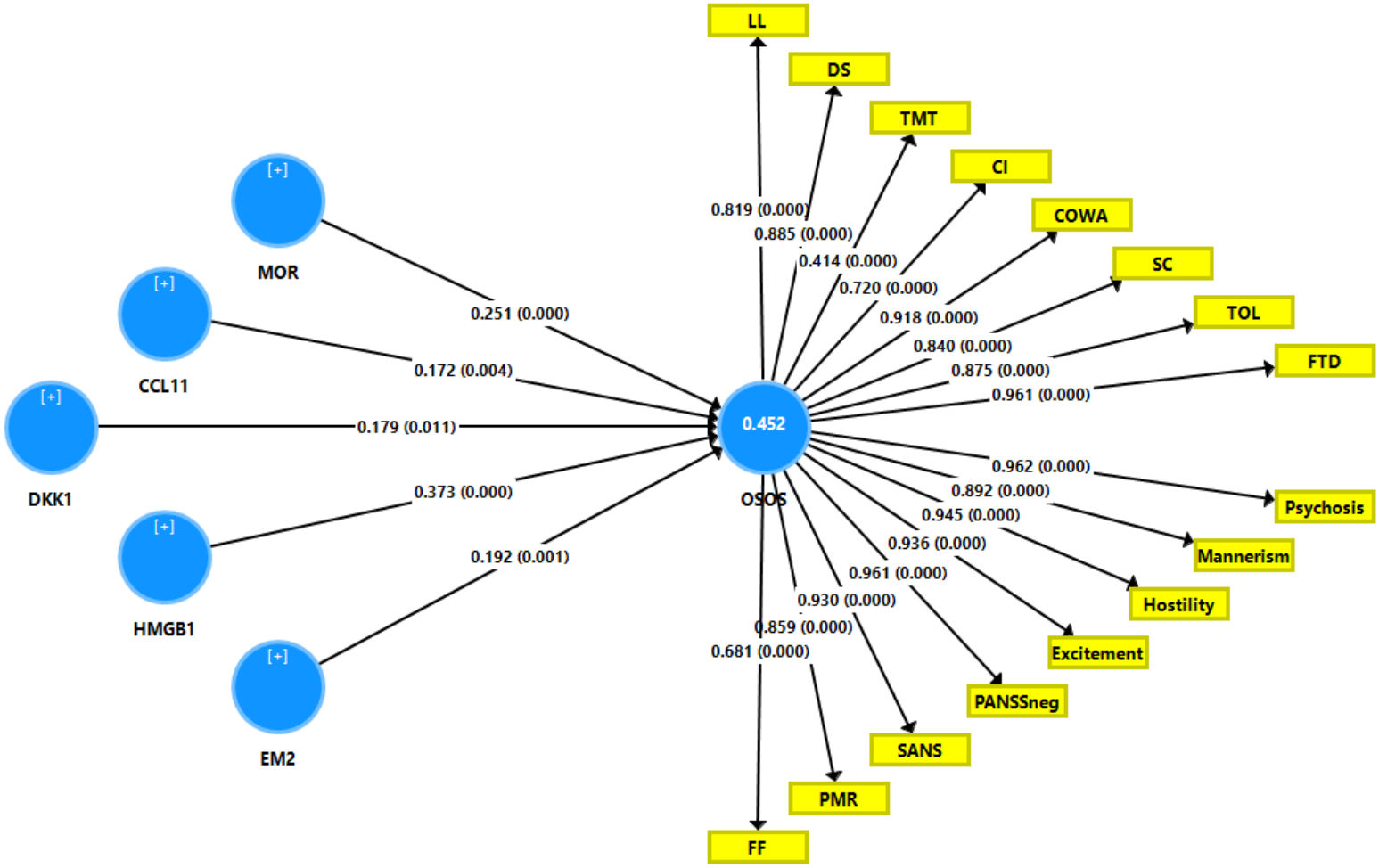

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Figure 4 Results of Partial Least Squares (PLS) path analysis with the biomarkers as input variables and symptom domains and neurocognitive tests results as output variables.

LL: List learning, DS: Digit Sequencing Task, TMT: Token Motor Task, CI: Category Instances, COWA: Controlled Oral Word Association Test, SC: Symbol coding, TOL: Tower of London, FTD: formal thought disorders.

FF: Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue, PMR: psychomotor retardation, SANS: Scale for the Assessment of Negative Symptoms, PANSSneg: negative subscales of the Positive and Negative Syndrome Scale.

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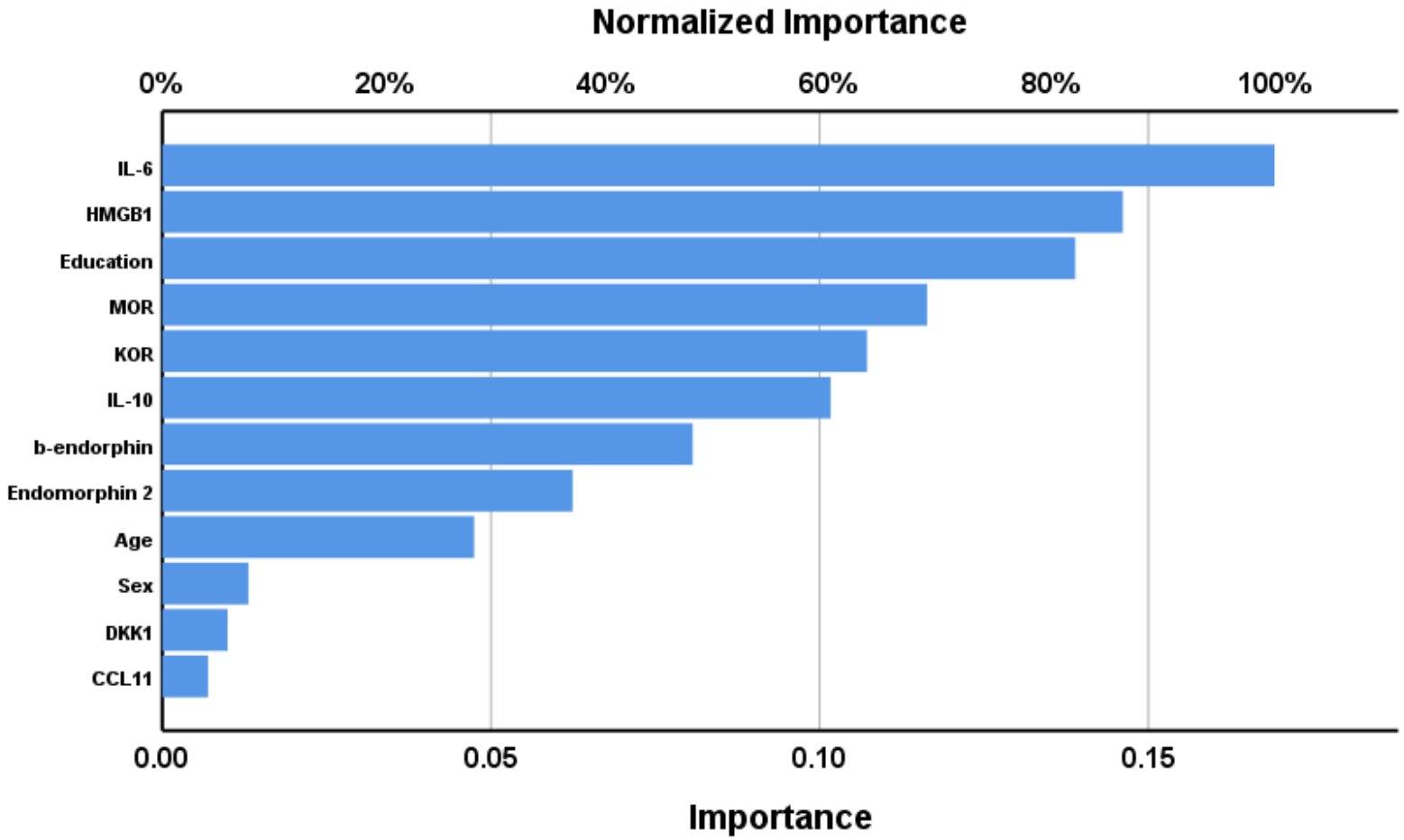

Figure 5 Results of a neural network showing the importance and relative importance of the biomarkers predicting the Fibromyalgia and Chronic Fatigue Syndrome Rating or FibroFatigue scale score. IL: interleukin, HMGB1: high mobility group box 1 protein, MOR: μ-opioid receptor, KOR: κ-opioid receptor, DKK1: Dickkopf protein 1, CCL11: CC-motif chemokine 11 or eotaxin.

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