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Serum Agrin and Talin, Two Muscular Proteins, Are Significantly Increased in Major Depression and Increased Agrin and Lowered Creatine Phosphokinase are Associated with Chronic Fatigue and Fibromyalgia Symptoms in Depression

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Abstract

Chronic fatigue and fibromyalgia symptoms frequently occur in major depressive disorder (MDD). The pathophysiology of these symptoms may in part, be ascribed to activated immune pathways, although it is unclear whether muscular factors play a role in their onset. The aim of the present study is to examine the role of muscle proteins in major depression in association with symptoms of chronic fatigue and fibromyalgia. We measured serum levels of agrin, talin-2, titin, and creatine phosphokinase (CPK) as well as the FibroFatigue (FF), the Hamilton Depression Rating Scale (HAM-D) and the Beck Depression Inventory (BDI-II) in 60 MDD patients and 30 healthy controls. The results show a significant increase in agrin and talin-2 in MDD patients as compared with controls. There were highly significant correlations between agrin and HAM-D, BDI-II and FF scores. Agrin, but not talin or titin, was significantly and positively associated with all 12 items of the FF scale. We found that a large part of the variance in HAM-D (47.4%), BDI-II (43.4%) and FF (43.5%) scores was explained by the regression on agrin, smoking, female sex (positively associated) and education (inversely associated). CPK was significantly and inversely associated with the total FF score and with muscle and gastro-intestinal symptoms, fatigue, a flu-like malaise, headache and memory, autonomic and sleep disturbances. These results suggest that aberrations in neuromuscular (NMJs) and myotendinous junctions may play a role in MDD and that the aberrations in NMJs coupled with lowered CPK may play a role in symptoms of chronic fatigue and fibromyalgia in MDD. Moreover, the increase of agrin in MDD probably functions as part of the compensatory immune-regulatory system (CIRS).

Keywords: major depression, chronic fatigue, fibromyalgia, neuro-immune, inflammation

Introduction

Fatigue or loss of energy are important symptoms of major depression (MDD) that affect work and normal life performance (DMS-IV). Chronic fatigue and physio-somatic symptoms including muscle pain and tension, gastrointestinal symptoms and a flu-like malaise are key symptoms of MDD (Maes, 2009). There is a strong comorbidity between MDD and chronic fatigue syndrome (CFS), an illness characterized by fatigue, muscle symptoms, fibromyalgic pain, gastro-intestinal symptoms and neurocognitive impairments (Maes et al., 2013). There is also a strong comorbidity between MDD and fibromyalgia, an illness characterized by widespread musculoskeletal pain coupled with affective and neurocognitive symptoms (Galek et al., 2015).

Interestingly, many patients with muscle related disorders have a high prevalence of depression (Wicks et al., 2007; Ferentinos et al., 2011). Furthermore, there are reciprocal associations between MDD and either low muscular strength or muscle weakness. For example, low muscular strength is independently associated with an increased risk to develop depression (Volaklis et al., 2019). MDD may be accompanied by an increased frequency of sarcopenia, pre-sarcopenia, low muscle mass, low muscle strength, and low muscle strength without loss of muscle mass (Heo et al., 2018; Szlejf et al., 2018). Bertoni et al. (2018) reported that the onset of depressive symptoms may impact muscle weakness (dynapenia). Furthermore, individuals with muscle weakness, measured as weak handgrip strength, have a higher prevalence

 doi:10.20944/preprints201906.0243.v1

of depression than those without this condition (McDowell et al., 2018; Ashdown-Franks et al., 2019).

Depression and CFS share common pathways, which may explain their comorbidity, including activated immune-inflammatory, neuro-oxidative and neuro-nitrosative pathways (Maes et al., 2013; Anderson et al., 2014). Moreover, patients with comorbid MDD and CFS show increased signs of neuro-oxidative and neuro-nitrosative stress as compared with MDD patients without CFS (Maes et al. 2012). Increased plasma concentrations of pro-inflammatory cytokines / chemokines including interleukin (IL)-6 and CXCL-8 (IL-8), and high-sensitivity C-reactive protein (hs-CRP) are reported in fibromyalgia in some (Backryd et al., 2017; Bazzichi et al., 2007; Ranzolin et al., 2016), but not all studies (Maes et al., 1999). Increased serum interleukin-6 and lactate are well established biomarkers of peripheral muscle fatigue (Finsterer et al., 2012) and both molecules may play a role in fatigue and muscle pain and weakness (Morris and Maes, 2017).

The production of muscle force involves a sequence of events, extending from cortical excitation to motor unit activation and excitation to contraction and muscle activation (Wan et al., 2017). Changes at any level in this pathway, including changes in the nervous, vascular and energy systems, impair force generation and contribute to the development of muscle fatigue (Wan et al., 2017). Early studies demonstrated that a change in muscle fibers and muscle enzymes, including creatine phosphokinase (CPK), are associated with psychiatric disorders including depression (Segal et al., 2007; Bălăiţă et al. 1990). Three major muscular molecules that control muscle functions are agrin, talin and titin. Agrin is produced by motoneurons and induces the aggregation of

 doi:10.20944/preprints201906.0243.v1

nicotinic acetylcholine receptors (nAChRs) (Hoch et al., 1994). Talin-2 is crucial for skeletal muscle development and regulates myoblast fusion, sarcomere assembly and the maintenance of myotendinous junctions (MTJs) (Conti et al., 2009). Talin-2 is expressed at high levels in skeletal muscle (Senetar and McCann 2005), and its expression is upregulated during myotube formation (Senetar et al., 2007). Ablation of the talin-2 gene leads to defects in the maintenance of MTJs and there is evidence that talin-1 and -2 mediate β1 integrin functions in myoblast fusion and sarcomere assembly (Conti et al., 2009). Titin, also known as connectin, is the third most abundant protein in muscles (after myosin and actin) and an adult human contains approximately 0.5 kg of titin (Guo and Sun 2018), the largest protein reservoir in the body (Frias-Soler et al., 2018). Titin's main function is the maintenance of myofibril assembly and the stabilization of sarcomere (Sun et al., 2014). Titin takes part in signaling (through a kinase domain), provides muscles with elasticity, allows post-contraction recovery, prevents overextension of muscle fibers and, as a consequence, plays a key role in the contraction of striated muscle tissues (Machado and Andrew 2000). Nevertheless, no studies have examined agrin, talin, titin and CPK in MDD in association with chronic fatigue and fibromyalgia symptoms.

Hence, the aims of this study is to examine a) serum levels of agrin, talin, titin and CPK in patients with major depression versus normal controls and b) whether their levels are associated with severity of depression, chronic fatigue and fibromyalgia symptoms. The a priori hypothesis is that depression is accompanied by lowered levels of these three proteins and increased CPK activity which could explain some of the chronic fatigue and fibromyalgia symptoms accompanying MDD.

 doi:10.20944/preprints201906.0243.v1

Subjects and Methods

Participants

In the current study we recruited 60 MDD patients aged 17-64 years and 30 age-matched healthy subjects as a control group. Participants were recruited at "The Psychiatry Unit", Al-Hakeem General Hospital, Najaf Governorate, Iraq during the period November 2018 till January 2019. The diagnosis was made using criteria of the 4th edition of Diagnostic and Statistical Manual of Mental Disorders, Text Revision (DSM-IV-TR) (APA, 2000). Patients were evaluated using a full medical history. We excluded a) subjects with systemic disease including autoimmune disorders, diabetes mellitus, inflammatory bowel disorder, COPD and chronic kidney disease; b) subjects with neurodegenerative or neuroinflammatory disease including multiple sclerosis, stroke, and Alzheimer and Parkinson's disease; and c) patients with other axis I diagnoses besides MDD including psycho-organic disorders, schizophrenia, OCD, substance abuse and CFS. None of the subjects practiced light sports regularly or any kind of heavy exercise. To eliminate any effects of overt inflammation, serum C-reactive protein (CRP) was evaluated in all samples and we excluded subjects with CRP values >6 mg/L. Written informed consent was obtained from all participants, according to the guidelines laid down in the current version of the Declaration of Helsinki, after approval from the ethics committee (IRB) of the College of Science, University of Kufa, Iraq (229-1/2017).

Measurements

Severity of depressive symptoms was assessed using the 24-item Hamilton Depression Rating Scale (HAM-D) one or two days before blood was drawn. BDI-II is a

 doi:10.20944/preprints201906.0243.v1

self-reported tool designed to determine the severity of depressive symptoms (Beck et al., 1996). The cutoff-scores were: 0–13 minimal, 14–19 mild, 20–28 moderate, and 29–63 severe. Severity of chronic fatigue and fibromyalgia symptoms was measured using the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (the FibroFatigue scale) (Zacchrisson et al., 2002). Body mass index (BMI) was measured by division of body weight in kilograms by the square of height in meters.

In the early morning hours, five milliliters of venous blood was drawn from patients and controls utilizing disposable needles and plastic syringes. The samples were transferred into a clean plain tube. 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 Eppendorf tubes to be stored at -80 °C until thawed for assay. Serum CRP was measured using a kit supplied by Spinreact®, Spain. This test is based on the principle of the latex agglutination. Commercial ELISA sandwich kits (Elabscience Co, China) were used to measure Agrin, Talin and Titin. Creatine phosphokinase activity in serum was measured spectrophotometrically using ready for use kit supplied by Spinreact®, Spain. We followed exactly all procedures according to the manufacturer's instructions. The intra-assay coefficients of variation (CV) of all assays were < 7.0%.

Statistical Analysis

Analysis of variance (ANOVA) was used to assess differences in continuous variables among diagnostic groups and analysis of contingency tables (χ^2-test) to assess associations between groups. Correlation among biomarkers and rating scales were computed using Pearson's product moment and Spearman's rank order correlation

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coefficients. Multivariate general linear model (GLM) analysis was used to assess the effects of diagnosis on biomarkers while controlling for confounders. Univariate GLM analyses were used to check the effects of explanatory variables on biomarkers. Consequently, model-generated (GLM analysis) estimated marginal mean (SE) values were computed. In addition, we z transformed all values of the biomarkers and displayed mean (SE) z values in cases and controls in bar plots. Multiple regression analysis was used to assess the biomarkers that predict HAM-D, FF scores and BDI-II score. All results were checked for multicollinearity using tolerance and VIF. We used binary logistic regression analysis to check predictors of major depression (dependent variable) and we computed Odd's ratios and 95% confidence intervals (CI). Using an ANCOVA with 3 groups and 5 covariates and a 2-tailed test at α=0.05 and assuming an effect size of 0.3 with power of 0.80, the a priori required sample seize was 90. All statistical analyses were performed using IBM SPSS windows version 25, 2017. Tests were 2-tailed and a p-value of 0.05 was used for statistical significance.

Results

Descriptive statistics

Table 1 shows the socio-demographic data as well as the raw values of the biomarkers used in this study**.** Patients were divided into those with **(**MDD+FF≥41**)** and without (MDD+FF<41) highly increased FF score using to the median split method. There were no significant differences in age, BMI and marital status among the three study groups. Patients with MDD+FF≥41 had a higher frequency of females, unemployment and urban living patients than controls. HAM-D and FF scores were

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significantly different among the three study groups, while the BDI-II score was significantly higher in patients versus controls. This Table also shows the raw measurements **(**not adjusted for extraneous variables**)** of the different biomarkers but we refer to Table 2 and 3 to evaluate the adjusted values in the study groups.

There were highly significant correlations between the HAM-A and BDI-II ($r=0.716$, $p<0.001$, $n=90$) and FF scores (0.842, $p<0.001$) and between BDI-II and FF scores ($r=0.842$, $p<0.001$). In the total study group, there were significant associations between agrin and talin ($r=0.462$, $p<0.001$, $n=90$) and CPK ($r=-0.289$, $p=0.006$), but not titin ($r=-0.019$, $p=0.858$).

Biomarker differences between controls and patients groups

Table 2 shows the outcome of a multivariate GLM analysis with all biomarkers as dependent variables and diagnosis (namely three groups: MDD+FF≥41; MDD+FF<41 and HC) while adjusting for age, sex, smoking and use of sertraline. The dependent variables were agrin, talin, titin, CPK and in addition the zA+zT composite score, namely a z unit weighted composite score computed as z transformation of agrin (z agrin) + z talin (reflecting activity of the NMJs and MTJs). We found a significant effect of diagnosis and use of sertraline on the dependent variables with effect sizes of 0.114 and 0.145, respectively. There were no significant effects of age, sex and smoking on the results. Also, there were no significant effects of BMI ($F=1.51$, $df=4/79$, $p=0.207$), education ($F=0.43$, $df=4/79$, $p=0.787$), use of citalopram ($F=0.77$, $df=4/79$, $p=0.550$) and use of fluoxetine ($F=0.90$, $df=4/79$, $p=0.466$).

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Table 2 shows also the results of univariate GLM analyses with the biomarkers as dependent variables and diagnosis and sertraline as explanatory variables. We found a significant association between all biomarkers (except Titin) and diagnosis with the strongest associations between diagnosis and agrin (effect size = 0.236) followed by zA+zT (effect size = 0.227). These associations remained significant after p correction for FDR, namely for agrin: p=0.0025; talin: p=0.024; CPK: p=0.024; and zA+zT: p=0.0025. **Table 3** shows the model-generated estimated marginal means of the biomarkers obtained by the univariate GLM analysis. **Figure 1** shows the differences in biomarker profile between the MDD and HC groups, with group mean values ±SE after z transformations were made. The results show that agrin is significantly higher in both MDD subgroups than in controls. Talin was significantly higher in MDD+FF≥41 than in healthy controls, while patients with MDD+FF<41 occupied an intermediate position. There were no significant differences in titin among the three study groups. The composite score was higher in both MDD subgroups than in controls.

Table 2 shows also that there were significant effects of sertraline use on agrin and the zA+zT score, while there were no significant effects on the other biomarkers. The effects on agrin (p=0.038) and the composite score (p=0.03) remained significant after p correction for FDR. Table 3 shows that use of sertraline increased agrin and the zA+zT score, but had no significant effect on the other biomarkers.

Table 4 shows the results of three binary logistic analyses with diagnosis of MDD as dependent variable and agrin, talin and zA+zT as explanatory variables while adjusting for relevant confounding variables**.** The results show that agrin yielded the greatest OR value (OR=7.56) followed by zA+zT (OR=4.79) and talin (OR=2.08).

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Table 5 shows the intercorrelation matrix (partial correlations adjusted for age, sex, sertraline use, BMI, smoking and education) between the biomarkers and severity of illness as measured with HAM-D, FF and BDI-II scores. The p values shown in the table were p corrected for FDR. There were significant associations between HAM-D, BDI-II and FF scores and agrin and the zA+zT composite score**.** HAM-D and FF score were significantly and inversely associated with CPK**.** There were no significant associations between any of the three clinical scores and titin**.** Table 5 shows also the significant (p corrected) correlations between agrin and the 12 FF items. There were no significant associations between talin and titin and any of the FF items. There were significant inverse associations between CPK and 9 FF items (all except concentration disorders, irritability, and sadness).

Table 6 shows the results of multiple regression analyses with the rating scale scores as dependent variables and the biomarkers and age, sex, smoking, antidepressant drug use, BMI, and education as independent variables. A significant part (43.5-47.4%) of the variance in symptoms domains was explained by the regression on agrin, female sex, smoking and education. **Figure 2** shows the partial regression plot with HAM-D score as dependent variable and agrin as explanatory variable (after adjusting for the variables shown in table 6). **Figure 3** represents the partial regression plot with FF score as dependent variable and agrin as explanatory variable.

Discussion

The first major finding of this study is that MDD is characterized by increases in the serum levels of the muscular proteins agrin and talin, while there are no significant

 doi:10.20944/preprints201906.0243.v1

changes in titin. In addition, agrin (but not talin or titin) is significantly associated with severity of depression as measured with the HAM-D and BDI-II scales. Agrin is a synapse organizing protein released by motor neuron axons at the NMJ and is utilized by motoneurons to induce AChR clustering and postjunctional differentiation thereby orchestrating differentiation, assembly and function of NMJs (McMahan 1990). Increasing levels of agrin may antagonize motor impairments and muscle atrophy (Boido et al., 2018). In addition, agrin is abundant in neural tissues, which modulate excitatory synaptic transmission, and in the microvasculature (Barber and Lieth, 1997; Wolburg et al., 2009). This protein plays a role in the formation and function of the blood brain barrier (BBB) (Wolburg et al., 2009) and reduced agrin in the brain may cause disruptions in BBB functions and synaptic loss (MacDonald et al., 2017). In patients with severe Alzheimer's disease agrin levels may be increased in the brain and be co-localized with beta-amyloid in senile plaques (Berzin et al., 2000). Two hypothetical mechanisms explaining increased serum agrin levels are: a) spillover of agrin outside the synaptic cleft; and b) damage to the BBB, which may occur in MDD (Patel and Frey, 2015).

High concentrations of talin 2 levels are observed in skeletal muscles (Senetar & McCann 2005) and in the brain (Senetar et al., 2005). The binding of talin, a major actin-binding protein, to integrin beta tails represents a final common step in integrin activation pathways (Calderwood, 2004). It is proposed that, in the brain, talin-2 binds with membrane molecules including phosphatidylinositol phosphate kinase type 1γ, and mediates synaptic vesicle trafficking and endocytosis (Ling et al., 2003; Morgan et al., 2004). Talin participates in the biology of periactive zones of synapses by regulating actin and clathrin coat dynamics (Morgan et al., 2004). Talin-2 concentrations are

 doi:10.20944/preprints201906.0243.v1

increased in cerebro-spinal fluid and decreased in serum in patients with epilepsy suggesting that talin-2 may be distributed between plasma and CSF compartments due to BBB leakage following seizures (Xiao et al., 2010). Acute stages of multiple sclerosis are accompanied by increased levels of talin-1 which may be explained by two mechanisms, namely leakage due to BBB disruption and increased extracellular transport of agrin-1 (Muto et al., 2017).

Nevertheless, agrin is also expressed by T cells and T cell activation is characterized by increased agrin levels as detected in systemic lupus erythematosus (Jury et al., 2007; Jury and Kabouridis, 2010). IL-1β or a cytokine mixture comprising IL-1β, tumor necrosis factor (TNF)-α and interferon (IFN)-γ may upregulate the agrin gene (Souza et al., 2004), whilst IFN-γ induces the expression of agrin in T cells via a STAT-3-related mechanism (Jury et al., 2007). Increased nitric oxide synthase (NOS) modulates talin expression (Tidball et al., 1999) and nitric oxide (NO) inhibits proteolysis of talin via S-nitrosylation (Koh and Tidball, 2000). By inference, increased production of these cytokines (Maes, 1995) and increased (S)-nitrosylation (Maes et al., 2011) in MDD may play a role in elevated agrin and talin levels in MDD. Therefore, our results do not allow to pinpoint the origin of increased agrin levels in MDD, although the significant associations between agrin and talin suggest that skeletal muscles at least in part contribute to increased agrin levels in MDD. Moreover, there were no significant differences in titin levels between patients and controls. The giant titin protein may be cleaved by calpain and matrix-metalloproteinase-2 when protease activity is increased in pathological conditions including oxidative stress (Beckendorf et al., 2015). Therefore, it is possible that any increases in titin in MDD may be blurred by effects of increased

 doi:10.20944/preprints201906.0243.v1

neuro-oxidative stress. Future research should examine whether the cleaved titin fragments are elevated in serum or urine (Rouillon et al., 2014; Kanda et al., 2017) of patients with MDD.

The second major finding of this study is that increased serum agrin levels were highly significantly correlated with the total FF score and with all 12 items of the FF scale, including muscle pain and tension but also with immune-related symptoms, such as a flu-like malaise. These results may suggest that aberrations in NMJs may play a role in the physio-somatic symptoms of MDD, including chronic fatigue and fibromyalgia. Alternatively, it is also possible that activated immune-inflammatory pathways are associated with increased agrin levels and elevated physio-somatic symptoms (Anderson et al., 2014) and thus explain in part the association between agrin and FF item scores.

The third major finding of this study is that CPK was significantly and inversely associated with agrin levels and FF symptoms including muscle and gastro-intestinal symptoms, fatigue, a flu-like malaise, headache and memory, autonomic and sleep disturbances. Importantly, CPK levels were significantly lowered in MDD patients with highly increased FF symptoms, but not in MDD patients with lower FF scores. Previously, it was shown that CPK levels are higher in mania than in depressive phases of bipolar disorder (Segal et al., 2007) and higher in non-psychotic major depression than in MDD with psychotic features (Segal et al., 2007). Bălăiţă et al. (1990) reported that CPK is higher in depressive, but not euthymic, MDD patients as compared with controls. Feier et al. (2011) found that CPK was not significantly different between depressive and euthymic bipolar patients. One study reported that fatigue ratings in an American Cup sailing team were significantly and inversely associated with CPK levels (Hadala et al.,

 doi:10.20944/preprints201906.0243.v1

2010). CPK catalyses the conversion of creatine to phosphocreatine, which functions as a stored energy reservoir that may quickly regenerate ATP, especially in cells with high energy requirements, including skeletal muscles and brain cells (Andres et al., 2008; Wyss and Schulze, 2002). As such, CPK is important for avoiding muscle fatigue at the onset of high-intensity stimulation, although during more prolonged stimulation, CPK may contribute to fatigue by increasing myoplasmic concentration of inorganic phosphate (Dahlstedt et al., 2001). Muscle fiber microruptures may cause increased outflow of CPK into the bloodstream and as a consequence increased levels may be used as a damage marker of tissues rich in CPK (Sorichter et al., 2001). Nevertheless, lowered serum CPK activity is observed in patients with severe infections and septicimeia and lowered CPK activity in those individuals may be explained by depleted levels of glutathione, a CPK preserving antioxidant (Gunst et al., 1998). Glutathione is significantly depleted in MDD and CFS (Maes et al., 2011; 2013) suggesting that lowered CPK in MDD with increased FF symptoms may be related to depletion of the glutathione system.

From the above discussion it may appear that elevated levels of agrin and talin have protective properties in skeletal muscles, BBB and brain cells. Moreover, there is now also evidence that agrin has negative immune-regulatory effects. Firstly, in macrophages and microglia, agrin modulates the cholinergic anti-inflammatory pathway that regulates inflammation (Mencel, 2014). Secondly, agrin significantly reduces IL-6 production in activated macrophages (Kabouridis et al., 2012). Thirdly, in macrophages, agrin augments the production of the anti-inflammatory cytokine IL-10 (Jury et al., 2007; Antoniv and Ivashkiv, 2011; Kabouridis et al., 2012). Furthermore, agrin-deficient monocytes exhibit low survival and are defective in phagocytosis (Mazzon et al., 2012).

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Talin may also play a role in the maintenance and survival of T regulatory cells and IL-2R expression (Klann et al., 2017). Based on reports that agrin expression is elevated by immune activation and in turn regulates immune-inflammatory pathways, we may suggest that the increase of agrin in MDD functions as part of the compensatory immune-regulatory system (CIRS), which down-regulates the primary immune-inflammatory response in MDD via multiple negative feedback systems (Maes, 1995; Maes and Carvalho, 2018).

The findings of this study should be interpreted with regard to its limitations. Firstly, this is a case-control study and therefore no inferences about causal relationships can be made. Secondly, it would have been more interesting if we had also measured pro-inflammatory cytokines and glutathione levels as well as indicants of nitrosylation in MDD. Thirdly, the use of antidepressants may affect serum levels of the analytes measured herein. For example, four weeks of paroxetine treatment increases creatine transport to the hippocampus and prefrontal cortex (Lugenbiel et al., 2010), while fluoxetine and escitalopram may alter CPK activity in hippocampus, striatum, and prefrontal cortex, albeit in different directions (Santos et al., 2009). In the present study no effects of antidepressant drugs were detected on CPK levels, although setraline treatment significantly increased agrin levels. Nevertheless, all results were adjusted for use of sertraline and other antidepressants.

Conclusions

MDD is associated with increased serum levels of agrin and talin-2, while agrin is significantly associated with severity of depression. Moreover, increased agrin levels and

lowered CPK levels are significantly associated with the severity of chronic fatigue and fibromyalgia scores in MDD. The overall results show that NMJ and MTJ proteins may participate in the pathophysiology of MDD and that agrin may additionally play a role as part of the CIRS in MDD.

Conflict of Interest

The authors declare no conflict of interest.

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Author's contributions

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

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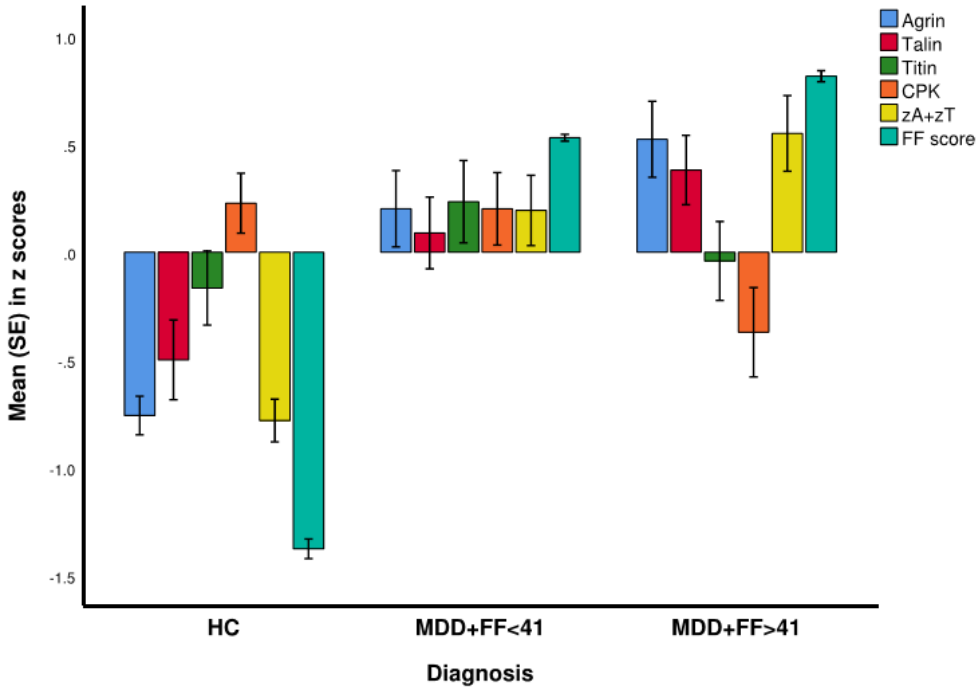

Figure 1 Differences in biomarker profile and FifroFatigue (FF) score between major depression (MDD) and healthy controls (HC), with group mean values ±SE after z transformations were made. Patients with MDD were divided into those with a highly increased FF scale score (MDD+FF≥41) versus those with a lower FF (<41) score.

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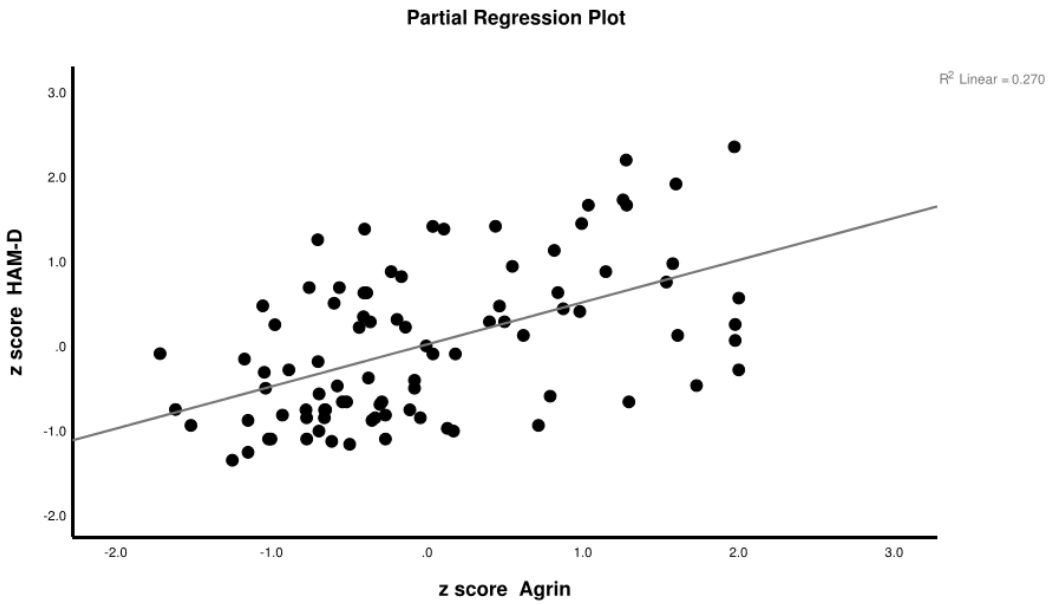

Figure 2 Partial regression plot with Hamilton Depression Rating Scale (HAM-D) score as dependent variable and agrin as explanatory variable.

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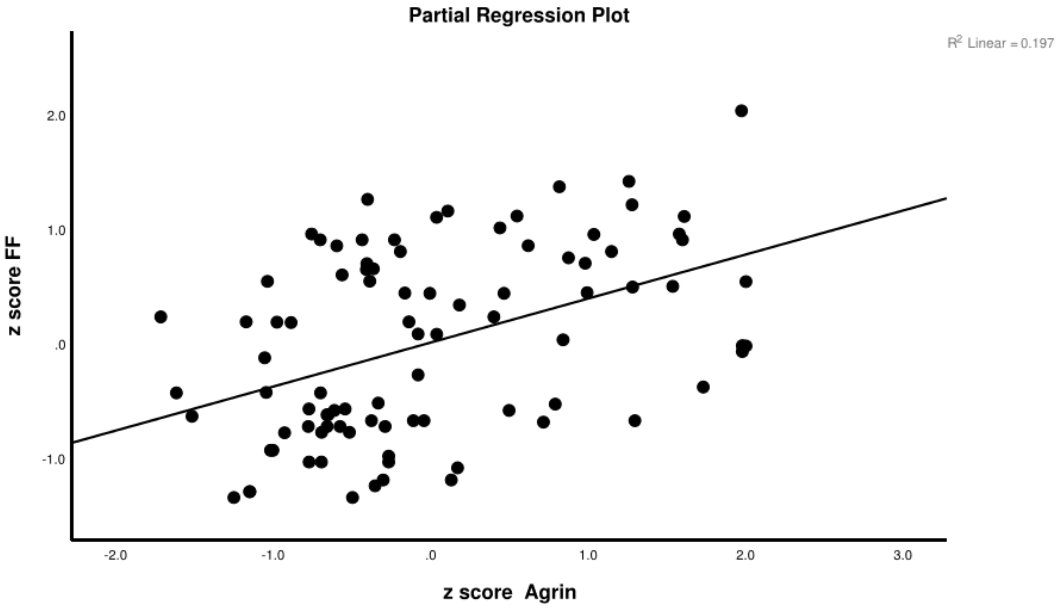

Figure 3 Partial regression plot with the FibroFatigue (FF) score as dependent variable and agrin as explanatory variable.

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Table 1. Socio-demographic and clinical data in major depressed patients with (MDD+FF≥41) and without (MDD+FF<41) highly increased FibroFatigue (FF) score
and healthy controls (HC)

Variables	HC [A] N=30	MDD+FF<41 [B] N=27	MDD+FF≥41 [C] N=33	F/Ψ/χ^2	df	p
Age (years)	37.7 (9.6)	34.6 (10.2)	38.5 (14.4)	0.87	2/87	0.423
Sex (F / M)	6/24[C]	7/20	19/14[A]	11.24	2	0.004
BMI (kg / m2)	26.4 (2.1)	25.3 (3.0)	25.8 (2.8)	1.25	2/87	0.292
Smoking (No / Yes)	30/0	19/8	22/11	0.368	-	0.002
Employment (No / Yes)	13/17[C]	20/7	28/5[A]	13.10	2	0.001
Education (literate / illiterate)	0/30	8/19	10/23	0.354	2	0.004
Married / Single	5/25	6/24	8/25	0.57	2	0.752
Rural / Urban	2/28[C]	4/23	12/21[A]	0.322	-	0.010
FF score	2.2 (1.5)[B,C]	37.0 (2.4)[A,C]	46.2 (5.1)[A,B]	KWT	-	<0.001
HAM-D score	1.9 (1.9)[B,C]	18.3 (5.4)[A,C]	23.7 (6.2)[A,B]	KWT	-	<0.001
BDI-II score	1. (1.1)[B,C]	18.5 (3.4)[A]	18.5 (2.6)[A]	KWT	-	<0.001
Agrin (ng/mL)	0.88 (0.81)[B,C]	2.46 (1.52)[A]	2.99 (1.66)[A]	19.30	2/87	<0.001
Talin (ng/mL)	1.73 (0.64)[B,C]	2.10 (0.54)[A]	2.28 (0.58)[A]	7.10	2/87	0.001
Titin (ng/mL)	2.52 (1.07).	2.97 (1.13)	2.71 (1.13)	1.19	2/87	0.309
Creatine Phosphokinase (U/L)	106.5 (22.8)[C]	105.7 (26.2)[C]	88.5 (35.7)[A,B]	3.82	2/87	0.026

Data are shown as mean (±SD).
[A,B,C]: pairwise comparisons between group means
FF: FibroFatigue scale; HAM-D: Hamilton Depression Rating Scale; BDI-II: The *Beck Depression Inventory-II*

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Table 2. Results of multivariate GLM analysis with biomarkers as dependent variables and diagnosis namely major depression (MDD) with a highly increased Fibrofatigue (FF) scale score versus those with a lower FF score and healthy controls as explanatory variable while adjusting for sex, age, smoking and use of sertralin.

Tests	Dependent variables	Explanatory variables	F	df	p	Partial η^2
Multivariate	All biomarkers	Diagnosis	2.58	8/160	0.012	0.114
		Sex	0.34	4/80	0.849	0.017
		Age	1.33	4/80	0.268	0.062
		Smoking	1.45	4/80	0.226	0.068
		Sertralin	3.39	4/80	0.013	0.145
Univariate	Agrin	Diagnosis	13.27	2/86	<0.001	0.236
	Talin	Diagnosis	4.36	2/86	0.016	0.092
	Titin	Diagnosis	0.69	2/86	0.506	0.016
	CPK	Diagnosis	4.16	2/86	0.019	0.088
	zA+zT	Diagnosis	12.63	2/86	<0.001	0.227
Univariate	Agrin	Sertralin	6.16	1/86	0.015	0.067
	zA+zT	Sertralin	7.79	1/86	0.006	0.083

CPK: creatine phosphokinase

zA+zT: z unit weighted composite score computed as z transformation of agrin (z A) + z talin (zT)

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Table 3 Model-generated estimated marginal means (as z scores) of the different biomarkers in patients with major depression (MDD) with a highly increased Fibrofatigue (FF) scale score (MDD+FF≥41) versus those with a lower FF (<41) score and healthy controls (HC).

Variables	**HC [A] n=30**	**MDD+FF<41 [B] n=27**	**MDD+FF≥ 41 [C] n=33**
Agrin (z score)	-0.471 (0.189) [B, C]	0.318 (0.164) [A]	0.636 (0.149) [A]
Talin (z score)	-0.244 (0.213) [C]	0.194 (0.185)	0.482 (0.169) [A]
Titin (z score)	0.080 (0.227)	0.335 (0.198)	0.056 (0.180)
CPK (z score)	0.346 (0.224) [C]	0.250 (0.195) [C]	-0.324 (0.177) [A,B]
zA+zT (z score)	-0.423 (0.188) [B, C]	0.305(0.164) [A]	0.661 (0.149) [A]
Variables	No sertralin	Sertralin	
Agrin (z score)	-0.125 (0.098)	0.447 (0.204)	
zA+zT	-0.140 (0.098)	0.500 (0.203)	

[A,B,C]: pairwise comparison between group means. Results expressed as mean±SE.

zA+zT: z unit weighted composite score computed as z transformation of agrin (z agrin) + z talin + z titin

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Table 4. Results of binary logistic regression analysis with major depression as dependent variable (and controls as reference group) and biomarkers as explanatory variables.

	Dependent Variables	**Explanatory variables***	**B (SE)**	**W**	**df**	**p**	**OR**	**95% CI**
#1	MDD *vs.* HC	Agrin	2.023(0.531)	14.54	1	<0.001	7.56	2.67 – 21.39
		Education	-1.133(0.345)	10.82	1	0.001	0.32	0.16 – 0.63
#2	MDD *vs.* HC	Talin	0.732(0.294)	6.19	1	0.013	2.08	1.17 – 3.70
		Education	-1.112(0.302)	13.55	1	<0.001	0.33	0.18 – 0.60
#3	MDD *vs.* HC	zA+zT	1.567 (0.418)	14.03	1	<0.001	4.79	2.11 – 10.88
		Education	-1.097 (0.336)	10.63	1	0.001	0.33	0.17 – 0.65

OR: Odds ration, 95% CI: 95% confidence intervals

zA+zT: z unit weighted composite score computed as z transformation of agrin (z agrin) + z talin

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Table 5. Intercorrelation matrices (partial correlations) between biomarkers and severity of illness

Variables	HAM-D	BDI-II	FF
Agrin	0.527**	0.353**[*]	0.426**
Talin	0.251	0.150	0.190
Titin	-0.033	-0.041	-0.074
CPK	-0.284*	-0.153	-0.261*
zA+zT	0.473**	0.305**	0.374**
Symptoms of FF	**Agrin**	**Talin**	**CPK**
FF1 Muscle pain	0.410**	0.144	-0.272*
FF2 Muscle tension	0.403**	0.131	-0.248*
FF3 Fatigue	0.355**	0.138	-0.233*
FF4 Concentration disorders	0.386**	0.203	-0.211
FF5 Memory disturbances	0.405**	0.206	-0.252*
FF6 Irritability	0.341**	0.112	-0.211
FF7 Sadness	0.368**	0.172	-0.208
FF8 Sleep disorders	0.451**	0.191	-0.242*
FF9 Autonomic disturbances	0.420**	0.211	-0.259*
FF10 Gastro-intestinal symptoms	0.440**	0.159	-0.252*
FF11 Headache	0.376**	0.248	-0.260*
FF12 Flu-like malaise	0.407**	0.240	-0.287*

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Shown are partial correlation coefficients with age, sex, body mass index, smoking, education and use of sertraline as covariates

*. Correlation is significant at the p=0.05 level (2-tailed). ** Correlation is significant at the p=0.01 level (2-tailed); all after p correction for false discovery rate

FF: FibroFatigue scale; HAM-D: Hamilton Depression Rating Scale; BDI-II: The *Beck Depression Inventory-II*

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Table 6. Results of multiple regression analyses with severity of illness scores as dependent variable and biomarkers as explanatory variables while adjusting for possible confounder variables

Dependent Variables	Explanatory variables	B (SE)	t	p	R^2	Model F	df	p
#1. HAM-D	Model				0.474	19.18	4/85	<0.001
	Agrin	0.371 (0.091)	4.10	<0.001				
	Smoking	0.878 (0.220)	3.99	<0.001				
	Female sex	0.386 (0.176)	2.22	0.031				
	Education	-0.169 (0.079)	-2.14	0.036				
#2 BDI-II	Model				0.434	9.65	4/85	<0.001
	Agrin	0.276 (0.094)	2.94	0.004				
	Education	-0.289 (0.082)	-3.52	0.001				
	Female sex	0.544 (0.183)	2.97	0.004				
	Smoking	0.650 (0.228)	2.85	0.006				
#3. FF	Model				0.435	15.75	4/82	<0.001
	Agrin	0.313 (0.095)	3.31	0.001				
	Education	-0.274.(0.084)	-3.38	0.002				
	Smoking	0.660 (0.229)	2.87	0.005				
	Female sex	0.450 (0.187)	2.41	0.018				

All scale variables are processed as z scores

FF: FibroFatigue scale; HAM-D: Hamilton Depression Rating Scale; BDI-II: The *Beck Depression Inventory-II*