Multiple Sclerosis and Related Disorders 63 (2022) 103846

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Cerebrospinal fluid amyloid precursor protein as a potential biomarker of fatigue in multiple sclerosis: A pilot study

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ARTICLE INFO

Keywords:
Fatigue
Amyloid precursor protein
MS
Biomarker
Insomnia
Neuropentraxin-1, FSS
ISI

ABSTRACT

Background: Fatigue is the major cause of disability in MS. Fatigue has been suggested to be primary, part of the neurological disease; it can also be secondary to other diseases outside the CNS or exist as a separate comorbidity. The only forms of measurement currently available are through subjective standardized questionnaires, which are not able to identify primary MS-related fatigue. Therefore, there is a need for objective biomarkers of fatigue in MS. This study explored the viability of 17 possible biomarkers of primary fatigue in MS. Our chosen biomarker panel represents the function and health of different parts of the CNS.

Methods: We evaluated 31 MS patients and 17 healthy controls using the Fatigue Severity Scale (FSS) and Insomnia Severity Index (ISI). We assessed clinical parameters and collected CSF from all participants to analyze 17 biomarkers, some of which in multiple targeted sequences, reflecting structural and functional changes in the brain. Based on FSS scores, MS was divided into MS-Fatigue (MS-F, FSS $\geq$ 4) and MS-NoFatigue (MS-NoF, FSS $<$ 4).

Results: MS-F had significantly lower levels of amyloid precursor protein (APP) peptides than MS-NoF ($p = 0.005$, $p = 0.011$). The only biomarker correlating with FSS in any group was APP in MS (r = -0.47, -0.52; $p = 0.007$, 0.002). APP did not correlate with any clinical parameter in MS but correlated with multiple markers. In MS, FSS correlated with the ISI and months since diagnosis.

Conclusion: Although the mechanisms remain unknown, altered APP metabolism in MS seems to be associated with fatigue. APP should be evaluated as a biomarker of the role of structural MS pathology in the development of fatigue in individual MS patients.

1. Introduction

Fatigue, which is persistent and extreme tiredness, weakness, or exhaustion (mental and/or physical), is one of the most commonly reported symptoms in almost all CNS diseases (Mills and Young, 2011; Dittner et al., 2004). In MS, fatigue has been reported to be a common symptom (Lerdal et al., 2007) and the major cause of impaired ability to work in international studies (Smith and Arnett, 2005; Hillert and Stawiarz, 2015). Fatigue is a multilayered condition and disentangling types of fatigue in a patient is often difficult. Fatigue has been suggested to be primary, part of the neurological disease; it can also be secondary to other diseases outside the CNS or exist as a separate comorbidity (Penner and Paul, 2017). Several standardized questionnaires have been validated for clinical assessment and diagnosis of fatigue in MS (Dittner et al., 2004). However, fatigue as a clinical symptom can present similarly regardless of cause, and no concrete criteria separating types of fatigue or differentiating fatigue from physiological tiredness and exhaustion have been established. Therefore, there is a need for objective biomarkers of fatigue in MS and other brain disorders. However, no biomarkers that stratify or predict fatigue have been identified (Penner

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https://doi.org/10.1016/j.msard.2022.103846
Received 28 October 2021; Received in revised form 15 April 2022; Accepted 1 May 2022
Available online 6 May 2022

and Paul, 2017). A multitude of different potential biomarkers have been investigated, including orexin (hypocretin-1) (Papuć et al., 2010), inflammatory cytokines (Hakansson et al., 2019), neurodegenerative markers (Hakansson et al., 2019), and components of the hypothalamic–pituitary–adrenal (HPA) axis (Gottschalk et al., 2005). In addition, some studies have indicated that amyloid-related proteins may be important in MS (Matias-Guiu et al., 2016; Augutis et al., 2013). We build on these studies and include biomarkers reflecting structural and functional changes in the brain, including amyloid-related processes.

This study explored the viability of 17 possible biomarkers of fatigue in MS. Our chosen biomarker panel represents the function and health of different parts of the CNS. We analyzed seven types of synaptic markers (SCG2, CHGA, VGF, NPTX1, neurogranin, SYT1, and SNAP25), two types of amyloid-related markers (Aβ42 and APP), two types of tau-related markers that represent Alzheimer-related neuronal dysfunction (tTau and pTau), one marker reflecting general neurodegeneration (NFL), three types of lysosomal and proteasomal markers (CTSF, GM2A, and Ub), one type of endosomal vesicle-recycling marker (AP2B1), and one marker involved in sleep-wake regulation (orexin-A). In addition, we compared the biomarker profiles of fatigued MS patients to those of non-fatigued MS patients and a control group. As insomnia correlates closely with fatigue (Johansson et al., 2021), we correlated all biomarkers to a standardized insomnia questionnaire.

2. Materials and methods

2.1. Subjects

We included 31 MS patients fulfilling the 2010 McDonald criteria (Polman et al., 2011) at the Department of Neurology of Sahlgrenska University Hospital in Gothenburg, Sweden, between February and August 2018. Simultaneously, 17 healthy controls (HCs) were recruited from the local community. Three MS patients had secondary progressive MS (SPMS) and 28 patients had relapsing remitting MS (RRMS) (Lublin et al., 2014). General inclusion criteria were no fatigue originating before MS diagnosis and no concurrent confounding disorders, such as other neurological diseases or severe psychiatric disorders. No patients treated with corticosteroids in the last 30 days were included. Seven MS patients were treated with central stimulants ($n = 4$ modafinil 100 mg, $n = 2$ amphetamine 10 mg, and $n = 1$ amantadine 200 mg). All patients and HCs participated in the study voluntarily and provided written consent. This study was performed in accordance with the Declaration of Helsinki and the Universal Declaration of Human Rights. The study was approved by the Swedish Ethical Review Authority in Gothenburg (DNR 223-15).

2.2. Magnetic resonance imaging

A standard MRI protocol for MS including intravenous gadolinium (Gd) contrast was performed on a 1.5 or 3 Tesla MRI scanner and included T1, T2, and fluid attenuation inversion recovery (FLAIR) sequences according to the Swedish guidelines (Vagberg et al., 2017). MRI was performed in association with diagnostic and neurological examinations a median 19.5 days afterwards. Information on the lesions was collected from the patients' journals. The number of lesions at inclusion and number of Gd-enhancing lesions were counted. The patients performed at least one new MRI within a year after inclusion, and new lesions in that period were noted.

2.3. Clinical assessment and specimen sampling

Patients were assessed by clinical neurological examination and disability scored using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). Fatigue was assessed using the Fatigue Severity Scale (FSS) (Krupp et al., 1989), which was the mean score of nine items with the cut-off for fatigue being ≥4 (Valko et al., 2008). The MS population was divided into groups of fatigued (MS-F, when FSS ≥ 4) and non-fatigued (MS-NoF, when FSS < 4) to facilitate the comparison of biomarker profiles. Sleep was assessed using the Insomnia Severity Index (ISI) (Bastien et al., 2001). Scores from the seven items are added together, and the cut-off value indicating insomnia ≥10 (Morin et al., 2011). The ISI was chosen for its high level of validation (Morin et al., 2011).

Lumbar puncture was performed according to the procedures recommended in the consensus protocol of the BioMS-EU network for CSF research in MS (Teunissen et al., 2009). CSF samples were transported on ice, and the first 12 mL of CSF was carefully mixed. After centrifugation, fractions were snap-frozen within 2 h in 0.5 mL aliquots and stored at −80 °C until analysis.

2.4. CSF biomarkers

The neurofilament light (NfL) concentration in the CSF was measured using an in-house enzyme-linked immunosorbent assay (ELISA) as described previously (Gaetani et al., 2018). CSF Aβ1-42, T-tau, and P-tau181 concentrations were measured using commercially available INNOTEST ELISAs (Fujirebio Europe, Ghent, Belgium). The orexin-A concentration was measured using an in-house radioimmunoassay (RIA) as described previously (Portelius et al., 2014). The neurogranin concentration was also measured using an in-house ELISA described previously (Kvartsberg et al., 2019). The SNAP-25 and synaptotagmin-1 concentrations were measured using a combination of enrichment with immunoprecipitation and quantitation by selected reaction monitoring mass spectrometry (SRM-MS) (Tible et al., 2020). SRM-MS was also used to directly quantify a panel of synaptic and lysosomal proteins in digested CSF ; AP-2 complex subunit beta (AP2B1 amino acids 712–719 and 835–842); amyloid precursor protein (APP 289-301 and 439-450); chromogranin-A (CHGA 194–213, 216–226, and 400–412); cathepsin-F (CTSF 103-116 and 442-450); GM2 ganglioside activator (GM2A 89–96 and 170–179); neuropentraxin-1 (NPTX1 144–152 and 386–400); secretogranin-2 (SCG2 58–66 and 593–601); Neurosecretory protein VGF (VGF 64–80 and 268–278); ubiquitin (Ub 12-27 and 64-72) (Sjödin et al., 2019; Brinkmalm et al., 2018).

2.5. Statistical analysis

Statistical analyses were performed in SPSS Statistics for Windows, Version 27.0 (IBM, Armonk, NY) and GraphPad Prism version 8.20 (GraphPad Software, San Diego, California USA). Because the distribution of many analyzed markers was not Gaussian and contained multiple ties, non-parametric analyses were performed. As the aim of this study was to investigate whether the concentration of any biomarker was significantly different in MS-F compared to MS-NoF and HCs, and not to compare HCs and MS-NoF, the non-parametric Mann-Whitney U-test was used for analysis of group differences in two pairs. Correlation matrices was produced in Prism.

Some markers were analyzed in multiple targeted sequences. When correlation analyses showed significance in all sequences of the same marker, Bonferroni correction for multiple analysis was not performed. We found it highly unlikely that all sequences of one marker would correlate significantly and similarly by chance. Significant single-sequence correlations are not presented unless they passed Bonferroni correction corresponding to the number of distinct markers analyzed ($n = 17$).

3. Results

The patients in the two MS groups were older than the HCs but similar in most parameters (Table 1). Some differences were observed in the months since diagnosis, with MS-NoF being diagnosed significantly more recently. The HC and MS-F groups had similar sex distribution, whereas the MS-NoF group had a somewhat larger proportion of men.

Table 1
Patient characteristics and descriptive statistics.

Characteristic	MS	MS-Fatigue	MS-No Fatigue	Healthy Controls	*p*
Number of participants	31	19	12	17	
Age, years	38.6 ± 11.1	38.5 ± 11.7	38.8 ± 10.7	26.9 ± 5.5	MSF-HC $p = 0.002$ MS-HC $p < 0.001$
Female	17 (55%)	12 (63%)	5 (45%)	11 (65%)	
FSS score	4.23 ± 1.67	5.41 ± 0.63	2.38 ± 0.91	2.75 ± 1.10	MSF-MSnoF $p < 0.001$ MS-HC p = 0.003 MSF-HC $p < 0.001$
Fatigued, >4 on FSS	19 (61%)	19 (100%)	0 (0%)	2 (12%)	
No. of patients using central stimulants	7 (23%)	7 (36%)	0 (0%)	0 (0%)	
ISI	10.5 ± 5.3	12.7 ± 4.7	7.0 ± 4.6	4.8 ± 5.4	MSF-MSnoF $p = 0.006$ MS-HC $p = 0.001$ MSF-HC $p < 0.001$
EDSS score	2.2 ± 1.3	2.3 ± 1.4	2.2 ± 1.3		
Months since relapse	56 ± 86	56 ± 73	55 ± 106		
Months since onset relapse	72 ± 94	80 ± 86	59 ± 108		
Months since diagnosis	39 ± 60	57 ± 70	12 ± 24		MSF-MSnoF $p = 0.018$
No. of T2 lesions, MRI brain n underwent MRI	12 ± 731	10 ± 7 19	14 ± 6.12		
No. of T2 lesions, MRI spine n underwent MRI	4 ± 614	4 ± 611	5 ± 53		
No. of patients with contrast enhancing lesions on MRI	2	0	2		

Data are given as n (%) or mean ± SD unless otherwise noted. Abbreviations: FSS, Fatigue Severity Scale; ISI, Insomnia Severity index; EDSS, Expanded Disability Status Scale; SD, standard deviation; MSF, MS-Fatigue; MSnoF, MS-No Fatigue; HC, healthy control. The Mann-Whitney U test was used. P-values are only reported for significant group differences.

We found significant correlations and differences in concentration levels (Tables 2 and 3). A full representation of the correlation results is provided in Supplementary Fig. 1. MS-F had significantly lower levels of APP than MS-NoF and HCs (Fig. 1). Both MS-F and MS had significantly higher levels of NFL than HCs (Table 2). The correlation analysis (Table 3) revealed that APP was the only biomarker correlating with the FSS in any group, correlating significantly in MS (APP_439 and APP_289 r = -0.47 and -0.52; $p = 0.007$ and 0.002, respectively). APP did not correlate with any clinical parameter other than fatigue, but exhibited a moderate correlation to multiple analyzed markers (Supplementary Fig. 1). In MS, FSS correlated with ISI and months since diagnosis. In MS-F, only the number of lesions on MRI of the brain and spine correlated with the FSS (Table 3).

Table 2
Significant group differences in biomarker concentrations.

Analyzed marker	MS-F	MS-NoF	HC	*p*
APP_439 median IQR	1.12 1.03-1.81	2.08 1.61-2.57	2.05 1.42-2.61	MSF-MSNoF $p = 0.005$, MSF-HC $p = 0.044$
APP_289 median IQR	0.40 0.37-0.62	0.61 0.52-0.70	0.61 0.43-0.74	MSF-MSNoF $p = 0.011$, MSF-HC $p = 0.09$
NFL median IQR	467 297-791	560 351-1779	297 170-416	MSF-HC $p = 0.015$

Abbreviations: MS-F, MS-Fatigue; MS-NoF, MS-No Fatigue; HC, healthy control; APP, amyloid precursor protein; IQR, interquartile range. The Mann-Whitney U-test was used.

Table 3
Significant correlations between neurochemical markers, clinical parameters, and questionnaire scores.

Variable	Group	Scale	r	*p*	*p**
APP (289, 439)	MS	FSS	-0.47, -0.52	0.007, 0.002	N/A
NPTX (144, 386)	MS	FSS	-0.48, -0.29	0.006, 0.12	ns
Orexin	MS	ISI	-0.33	0.069	ns
Scg_2 (58, 593)	HC	ISI	0.49, 0.48	0.045, 0.050	N/A
ISI	MS	FSS	0.60	< 0.001	N/A
Months since diagnosis	MS	FSS	0.51	0.003	N/A
No. MRI-brain lesions	MS-F	FSS	0.60	0.006	N/A
No. MRI-spine lesions	MS-F	FSS	0.62	0.043	N/A

* Bonferroni corrected, applied for single significant fragments ns, not significant after Bonferroni correction

N/A, not applicable –Bonferroni correction was not applied when a significant correlation was found in all fragments. Clinical parameters were not Bonferroni corrected. Abbreviations: FSS, Fatigue Severity Scale; ISI, Insomnia Severity Index; HC, healthy control; APP, amyloid precursor protein; NPTX, neuropentraxin; Scg_2, secretogranin 2.

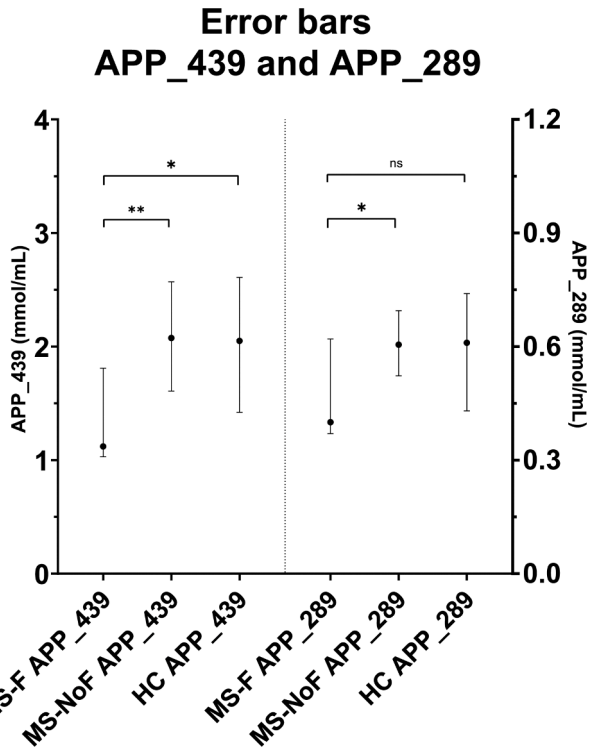

Fig. 1. Concentration of the amyloid precursor protein (APP) fragments by group. APP_439 and APP_289 indicate which APP fragment is presented. Abbreviations: MS-F, MS-Fatigue; MS-NoF, MS-NoFatigue; HC, healthy control; ns, no significance. Data are presented as median values and IQRs. * $p < 0.05$, ** $p < 0.01$.

3.1. Group differences in biomarker levels

The concentration levels of both monitored APP sequences were significantly lower in MS-F than MS-NoF (APP_439 $p = 0.005$, APP_289 $p = 0.011$; Fig. 1 and Table 2). Moreover, the concentration of APP_439 was significantly lower in MS-F than in HCs ($p = 0.044$). Aside from APP,

only NFL had significantly different concentration levels in MS-F and HCs. When comparing MS-F and MS-NoF, no significant difference was found in NFL; only APP exhibited a significant difference in the analyzed markers.

3.2. Biomarkers and fatigue

We performed correlation analyses between MS patients and HCs in order to investigate whether any potential biomarkers correlated with the FSS or ISI (Table 2). As seen in Fig. 2 and Table 3, both APP sequences inversely correlated with the FSS in the MS group (APP_439: r = -0.52, $p = 0.002$; APP_289: r = -0.47, $p = 0.007$). Linear regression analysis showed that the two APP sequences could explain 26% and 22%, respectively, of the variance in the FSS score in the MS group. One NPTX sequence correlated with the FSS but did not pass Bonferroni correction. In MS-F, no biomarker correlated with the FSS or ISI. Orexin did not correlate with the FSS but had borderline significant correlation with the ISI before Bonferroni correction. In the HC group, no markers correlated with the FSS after Bonferroni correction for single sequence. However, SCG-2 had a borderline significant positive correlation with the ISI (Table 3).

3.3. Correlation of APP with other factors

We investigated why the MS-F group had significantly lower levels of APP than the MS-NoF and HC groups through correlation analyses. APP did not correlate with any investigated clinical factors, including disability (i.e., EDSS), age, date or time of day for lumbar puncture, disease time parameters, inflammatory cells in the CSF, or number of lesions on MRI of the brain or spine. However, APP correlated with most other analyzed biomarkers to some degree (Supplementary Fig. 1). Aside from the two sequences that strongly correlated with each other, which is consistent with them both belonging to soluble APP (sAPP) (Fig. 2), the strongest correlations were seen with NPTX (APP_439-NPTX (144, 386): r = 0.87 and r = 0.69, $p < 0.001$; APP_289- NPTX (144, 386): r = 0.83 and r = 0.73, $p < 0.001$).

3.4. Clinical parameters

The MS-F and MS-NoF groups were very similar in all clinical parameters (Table 1). Other than the FSS score, only ISI and months since diagnosis significantly separated the groups. In MS, FSS correlated (Table 3) with the ISI (r = 0.60, $p < 0.001$) and months since diagnosis (r = 0.51, $p = 0.003$). In MS-F, FSS correlated with the number of lesions on MRI of the brain (r = 0.60, $p = 0.006$) and spine (r = 0.62, $p = 0.043$). Only two patients presented with contrast-enhancing MRI lesions, both of which were MS-NoF. EDSS did not correlate with the FSS in any group. The MS patients were significantly older than HCs, but age did not correlate with any parameter in HCs and only disease time parameters in MS. Analyzing all participants together revealed that age only correlates with NFL (r = 0.30, $p = 0.039$).

4. Discussion

The aim of this study was to find an objective biomarker that separates MS-F from MS-NoF and correlates with fatigue in MS. We found that APP levels are significantly lower in MS-F patients than MS-NoF patients and significantly lower than in HCs for one sequence and borderline significantly lower for the other. Moreover, we found an inverse correlation between FSS and APP in MS.

It is well established that APP accumulates in damaged axons (Mangiardi et al., 2011; Ferguson et al., 1997). Furthermore, APP has been proposed as a potential marker of axon demyelination and axonal injury (Moore et al., 2014). Mice studies have shown that APP plays a role in myelination, as APP knockout mice exhibit decreased myelin sheet thickness, while mice over-expressing APP exhibit an increased thickness (Xu et al., 2014). APP has been proposed to modulate nodular formation in axons (Xu et al., 2014) and is speculated to be co-expressed with proteins associated with neuroprotective properties (Angelov et al., 1998). Moreover, APP knockout mice presented with reduced brain weight and gliosis (Ring et al., 2007; Zheng et al., 1995), impaired synaptic plasticity associated with abnormal synaptic function in the hypothalamus (Seabrook et al., 1999), and impaired long term potentiation and spatial learning (Ring et al., 2007). In addition, recent studies show that APP seems to play an important role in regulating inhibitory neurotransmission (Kreis et al., 2021).

The only disease in which APP has been previously studied is Alzheimers Disease, where APP is well known to play a part in the pathogenesis. The connection of increased APP levels and dementia is underlined by the fact that triplications of APP in humans and overexpression of APP in mice have been associated with early onset of cognitive impairment (Kreis et al., 2021; Grangeon et al., 2021). However, to the best of our knowledge, this study is the first to find lower APP levels in a patient group, and the first to investigate the relationship of APP and fatigue in any disease.

Thus, more studies are needed in order to investigate whether lower

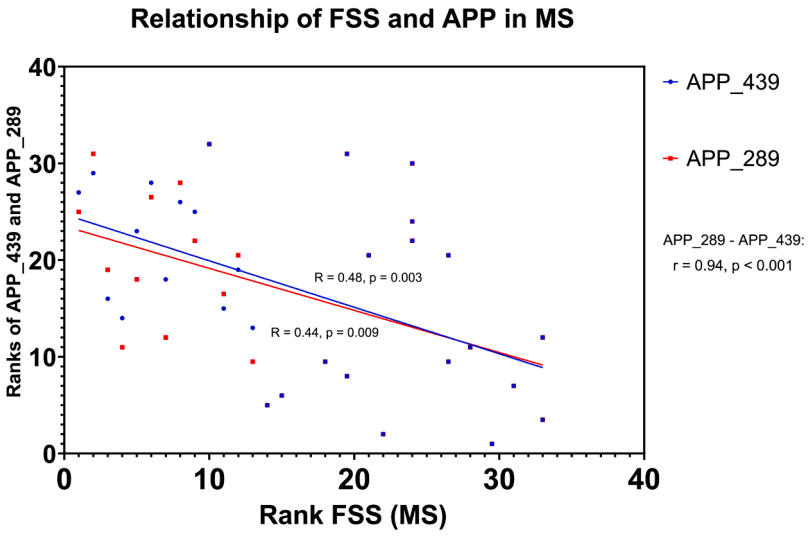

Fig. 2. Relationship between FSS and APP in MS. Non-parametric correlations of FSS and the APP fragments are shown along Spearman correlation coefficient R from the linear regression analysis. Abbreviations: FSS, Fatigue Severity Scale; APP, amyloid precursor protein.

APP levels is specific for MS fatigue. However, the proposed functions of APP seems to correspond well to present theories about structural and functional brain changes as the pathogenesis of fatigue in MS (Penner and Paul, 2017). The importance of APP in axonal myelination, formation and health further corroborates the hypothesis that lower APP levels might be specific for MS fatigue. Therefore, we propose that disrupted APP metabolism in fatigued MS patients is related to the development of fatigue. The direction of this association and the causality of our findings cannot be established. It is possible that MS patients with lower levels of APP might be more susceptible to processes leading to the development of fatigue. For example, reduced APP levels might expose MS patients to neural degeneration and subsequent brain atrophy, previously associated to fatigue (Penner and Paul, 2017). Another possibility is that neurodegeneration in MS might lead to loss of APP, which could lead to fatigue provided that lower levels of APP might cause symptoms identified as fatigue. Regardless, we suggest that APP might be used as a biomarker for fatigue in order to differentiate primary MS fatigue from other forms of fatigue.

Interestingly, the only conventional immunotherapy shown to significantly reduce fatigue in MS is natalizumab (Penner and Paul, 2017; Svenningsson et al., 2013). In an earlier study, we found that levels of sAPP were lower in MS patients and normalized after natalizumab treatment (Augutis et al., 2013). Supported by a previous study, where reintroduction of sAPP in APP knock-out mice counteracted reduced spatial learning and long term potentiation (Ring et al., 2007), our results suggest that restored APP levels may mediate the fatigue-alleviating effect of natalizumab.

In the current study, we found a clustered subset of MS-F patients with remarkably homogeneous levels of APP, whereas some MS-F patients presented with APP levels and distributions similar to that of MS-NoF (Fig. 1). As discussed previously, fatigue is a multifactorial symptom with many different underlying causes. Using the processes and tools available today, it is not possible to identify MS patients suffering from primary MS-related fatigue pre-inclusion. Therefore, defining a fatigue group by subjective fatigue is bound to include patients with different types of fatigue. Not all fatigued patients suffer from MS-related fatigue, so not all MS-F patients are expected to have altered levels of a biomarker reflecting possible MS-related structural or functional changes causing fatigue.

Thus, inclusion of multiple patients with non-primary fatigue in whom the process affecting the candidate biomarker is not present will render direct comparisons of concentrations on a group level less likely to yield results. This may be one reason that many earlier studies on biomarkers of fatigue did not find significant results (Papuć et al., 2010; Hakansson et al., 2019; Constantinescu et al., 2011). However, in this study, we found significantly different APP levels in MS-F and MS-NoF and borderline significant differences from HCs despite our study group likely containing patients with other forms of fatigue, thereby strengthening the notion that altered levels of APP may reflect a biological process related to fatigue in MS. This notion is further supported by the fact that APP correlates moderately with fatigue and exhibits a significant association, explaining 21–26% of the variance in fatigue in the MS group.

Clinically, many MS patients describe their lives as being negatively affected by fatigue. However, fatigue is attributed to any number of reasons other than MS, from psychological factors to physical inactivity, for many patients. Our findings support the patients' perspective that their fatigue is directly related to structural damage by the neurological disease. This finding can be used to support the claim that the patient's fatigue is a part of their neurological disease and a valid cause for not being able to work full-time. This is important because fatigue is the main cause of impaired ability to work (Smith and Arnett, 2005) and a common source of friction with employers and the social insurance system.

In this study, the only clinical parameters correlating to fatigue were disease time in MS and MRI lesion load in MS-F. However, the MS-F group did not have more MRI lesions than the MS-NoF group, and lesion load did not correlate with fatigue in all MS patients or MS-NoF patients. Contrast-enhanced lesions on MRI, indicating ongoing inflammatory activity, was not an important factor in MS fatigue; only two patients presented with contrast-enhanced lesions, both of which were MS-NoF. Thus, our findings are not conclusive regarding the role of MRI lesion load in MS fatigue, reflecting findings from other recent studies (Hakansson et al., 2019). However, it may not be lesion load itself, but rather lesion location, that is important in the development of MS fatigue (Altermatt et al., 2018). Therefore, our conflicting MRI results could be explained by different locations of the MRI lesions in MS-F and MS-NoF. However, lesion location was not investigated in this study.

This study did not find any significant correlation between orexin and fatigue, or any significant difference in orexin levels for MS-F and MS-NoF, contradicting some earlier findings (Papuć et al., 2010) but confirming others (Constantinescu et al., 2011). However, we did find a borderline significant correlation of orexin and ISI in MS, though it did not remain after Bonferroni correction. As fatigue has been tightly correlated with insomnia (Johansson et al., 2021), the tendency of an association between orexin and insomnia may give some clue regarding why some studies found that orexin and fatigue correlate while some did not.

5. Limitations

As this was a cross-sectional study, it is not possible to draw conclusions on the causality of our findings. In addition, because most of the included patients had RRMS, our findings may not be representative of SPMS. A majority of MS patients experience fatigue (Penner and Paul, 2017); therefore, the MS-NoF group was smaller than the MS-F group, which may affect the statistical analysis. The HC group was younger than the MS group, but age did not correlate with fatigue in any group in this study.

6. Conclusion

In this study, lower APP levels correlated with more fatigue in MS. MS-F patients had significantly lower levels of APP than MS-NoF patients and borderline significantly lower levels of APP than HCs. Thus, altered APP metabolism in fatigued MS patients may be connected to the development of fatigue. Moreover, normalization of APP by natalizumab may be a mediator of the fatigue-alleviating effect of natalizumab seen in earlier studies. Clinical measurement of APP levels in fatigued MS patients may be useful to support the notion that the patient's fatigue is a part of the neurological disease.

CRediT authorship contribution statement

Kalle Johansson: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Pontus Wasling:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Lenka Novakova:** Investigation, Writing – review & editing. **Simon Sjödin:** Data curation, Investigation. **Ann Brinkmalm:** Methodology, Writing – review & editing. **Gunnar Brinkmalm:** Methodology, Writing – review & editing. **Kaj Blennow:** Conceptualization, Methodology, Resources, Writing – review & editing. **Henrik Zetterberg:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Markus Axelsson:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Conflict of Interest

KJ, PW, SS, AB, GB has no conflict of interest. LN has received honoraria for lectures and/or advisory board membership from Biogen,

Novartis, Merck, and Sanofi Genzyme. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. HZ has served on scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. MA has received honoraria for lectures and/or advisory board membership from Biogen, Novartis, and Sanofi Genzyme.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding statement

This study was supported by research grants from the Göteborg Foundation for Neurological Research, the MS Research Foundation of the Gothenburg MS Society, the Edit Jacobsson Foundation Gothenburg, and NEURO Sweden. KB is supported by the Swedish Research Council (#2017-00915), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the National Institute of Health (NIH), USA, (grant #1R01AG068398-01), and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL.

Acknowledgements

The funding sources listed below had no influence on the decision to publish this article or the contents thereof. This study was supported by research grants from the Göteborg Foundation for Neurological Research, the MS Research Foundation of the Gothenburg MS Society, the *Edit Jacobsson* Foundation Gothenburg, and NEURO Sweden. KB is supported by the Swedish Research Council (#2017-00915), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the National Institute of Health (NIH), USA, (grant #1R01AG068398-01), and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla 375 Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2022.103846.

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