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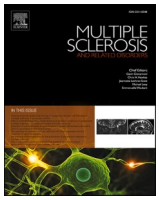

# BACE1 influences clinical manifestations and central inflammation in relapsing remitting multiple sclerosis

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ABSTRACT

Neurodegenerative and inflammatory processes influence the clinical course of multiple sclerosis (MS). The β-site amyloid precursor protein cleaving enzyme 1 (BACE1) has been associated with cognitive dysfunction, amyloid deposition and neuroinflammation in Alzheimer's disease.

We explored in a group of 50 patients with relapsing-remitting MS the association between the cerebrospinal fluid (CSF) levels of BACE1, clinical characteristics at the time of diagnosis and prospective disability after three-years follow-up. In addition, we assessed the correlations between the CSF levels of BACE 1, amyloid β (Aβ) 1-40 and 1-42, phosphorylated tau (pTau), lactate, and a set of inflammatory and anti-inflammatory molecules.

BACE1 CSF levels were correlated positively with depression as measured with Beck Depression Inventory–-Second Edition scale, and negatively with visuospatial memory performance evaluated by the Brief Visuospatial Memory Test-Revised. In addition, BACE CSF levels were positively correlated with Bayesian Risk Estimate for MS at onset, and with Expanded Disability Status Scale score assessed three years after diagnosis. Furthermore, a positive correlation was found between BACE1, amyloid β 42/40 ratio (Spearman's $r = 0.334$, $p = 0.018$, $n = 50$), pTau (Spearman's $r = 0.304$, $p = 0.032$, $n = 50$) and lactate concentrations (Spearman's $r = 0.361$, $p = 0.01$, $n = 50$). Finally, an association emerged between BACE1 CSF levels and a group of pro and anti-inflammatory molecules, including interleukin (IL)-4, IL-17, IL-13, IL-9 and interferon-γ.

BACE1 may have a role in different key mechanisms such as neurodegeneration, oxidative stress and inflammation, influencing mood, cognitive disorders and disability progression in MS.

## 1. Introduction

Multiple sclerosis (MS) is an autoimmune disease characterized by immune-mediated inflammatory processes affecting central myelin. However, converging evidence has shown that cortical damage and early neurodegeneration represent important pathophysiological features of MS. (Diker et al., 2016; Pravatà et al., 2017). Studies in animal models and in MS patients have indicated that neuronal and dendritic loss critically contributes to MS progression and plays a key role in cognitive dysfunction (Eshaghi et al., 2018; Pravatà et al., 2017). The pathogenesis of neurodegeneration in MS is multifactorial and several mechanisms may have a role, including inflammation, oxidative damage and altered amyloid homeostasis (Stampanoni Bassi et al., 2017). Better knowledge of mechanisms involved in neurodegeneration and the

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identification of specific biomarkers are crucial to preventing clinical worsening in MS.

The β-site amyloid precursor protein cleaving enzyme 1 (BACE1) has been identified as a prognostic marker in different neuro-psychiatric conditions (Mattsson et al., 2009; Stertz et al., 2018). Clinical studies in patients with Alzheimer's disease (AD) have confirmed the importance of BACE1 in the pathophysiology of the disease, pointing to BACE1 as a possible biomarker of disease progression. Accordingly, BACE1 has been directly associated with the risk of mild cognitive impairment (MCI) and conversion to AD (Zhong et al., 2007), and negatively correlated with cognitive performance (Mouton-Liger et al., 2020) and hippocampal volumes (Ewers et al., 2011). Increased BACE1 expression and activity in the cerebrospinal fluid (CSF) of AD patients has been associated with enhanced amyloid β (Aβ) synthesis and deposition (Cole and Vassar, 2007).

In addition to its role in amyloid regulation, BACE1 may influence several other processes under physiological and pathological conditions, including central and peripheral myelination, central nervous system (CNS) development, cognitive function, and synaptic plasticity (Hampel et al., 2021). Interestingly, it has been reported that BACE1 may also regulate neuroinflammation. In particular, BACE1 activity is modulated by activated microglia (Millot et al., 2020), suggesting a role in the crosstalk between neuroinflammation, Aβ homeostasis, and neurodegeneration (Hampel et al., 2021; Reiss et al., 2018).

To explore the role of BACE1 in MS, we investigated in a group of patients with relapsing-remitting (RR) phenotype the association between BACE1 CSF levels at the time of diagnosis and clinical characteristics, including clinical disability evaluated after 3 years of follow-up. In addition, we explored the association between BACE1 and CSF levels of Aβ 1-40 and Aβ 1-42, lactate, and concentrations of a large set of inflammatory molecules.

## 2. Materials and methods

### 2.1. MS patients

We enrolled a group of 50 consecutive RR-MS patients at the time of diagnosis. We admitted patients to the neurological clinic of the Neuromed Research Institute in Pozzilli, Italy, between 2017 and 2019. The diagnosis of MS was made on the basis of clinical, laboratory, and MRI parameters. The Ethics Committee of the Neuromed Research Institute in Pozzilli, Italy approved the study according to the Declaration of Helsinki (cod. 06-17). All patients gave written informed consent to participate in the study. At the time of diagnosis, patients underwent clinical evaluation, brain and spine MRI, and lumbar puncture (LP). Clinical characteristics recorded were age, sex, disease duration, expanded disability status score (EDSS) at LP. We also assessed the EDSS after three years of follow-up. Bayesian Risk Estimate for Multiple Sclerosis (BREMS) at onset, an individual risk score for the early prediction of long-term disease evolution, was calculated (Bergamaschi et al., 2015).

All the patients underwent 1.5T MRI scan of brain and spinal cord, which included the following sequences: dual-echo proton density, fluid-attenuated inversion recovery (FLAIR), T1-weighted spin-echo (SE), T2-weighted fast SE, and contrast-enhanced T1-weighted SE before and after intravenous gadolinium (Gd) infusion (0.2 mL/kg). Radiological disease activity at the time of LP was defined as the presence of Gd-enhancing (Gd+) lesions at the time of hospitalization in brain and spinal cord.

Fatigue was assessed using the fatigue severity scale (FSS), a nine-item questionnaire that measures the severity of fatigue symptoms on a seven-point scale (Krupp, 1989). We also measured visuospatial memory with the Brief Visuospatial Memory Test-Revised (BVMT-R) (Binzer et al., 2019). Mood was assessed using dedicated anxiety and depression scales. Beck Depression Inventory–Second Edition (BDI-II) was used to assess the presence of depressive symptoms (Sica, C., & Ghisi, 2007). This scale includes 21 items and the score range is from 0 to 63. A cutoff of 13 was used to detect depression ("The Goldman Consensus statement on depression in multiple sclerosis," 2005). Levels of anxiety were assessed using the State-Trait Anxiety Inventory form-Y (STAI-Y), a 40-item self-administered questionnaire exploring both the levels of situational anxiety (STAI-Y state) and the tendency to anxious situations (STAI-Y trait) (Pedrabissi, L., & Santinello, 1989).

The disease-modifying therapy (DMT) type, classified as first- or second-line treatment, was also recorded.

The control group comprised 25 patients with non-inflammatory/non-degenerative CNS or peripheral nervous system disorders, such as vascular leukoencephalopathy (N = 15) patients), metabolic and hereditary polyneuropathies (N = 9), normal pressure hydrocephalus (N = 1), pseudotumor cerebri (N = 1), functional neurological disorder (N = 2), migraine (N = 1), and spastic paraparesis (N = 1).

### 2.2. CSF collection and analysis

CSF was collected at the time of diagnosis, during hospitalization, by LP. No corticosteroids were administered before LP. Disease-modifying therapies were initiated after the confirmed diagnosis, when indicated. CSF was stored at -80°C and then analyzed using a Bio-Plex multiplex cytokine assay (Bio-Rad Laboratories, Hercules, CA, USA). CSF BACE1, Aβ 1-42 and 1-40, tau and phosphorylated tau (pTau) levels were measured by using Human ProcartaPlex multi-plex immunoassay kits (Thermo Fisher, Santa Clara, California), according to the manufacturer's instructions. Fluorescence intensity was measured with the Luminex 200 analyzer (Luminex Corporation, Austin, TX, USA) and data were analyzed using the xPonent 3.1 software (Luminex Corporation, Austin, TX, USA). CSF cytokines levels were determined according to a standard curve generated for the specific target and expressed as picograms/milliliter (pg/mL). Biochemistry assays were carried out using commercially available kits following the manufacturer's instruction. Samples were analyzed in triplicate. The CSF cytokines analyzed included: interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, tumor necrosis factor (TNF), interferon-gamma (IFN-γ), macrophage inflammatory protein 1α (MIP-1α), macrophage inflammatory protein 1β (MIP-1β), monocyte chemoattractant protein 1 (MCP-1), granulocyte colony-stimulating factor (G-CSF), interleukin-1 receptor antagonist (IL-1ra), and regulated upon activation, normal T cell expressed and secreted (RANTES).

### 2.3. Statistical Analysis

Shapiro–Wilk test was used to evaluate normality distribution of continuous variables. Data were shown as mean (standard deviation, SD) or median (interquartile range, IQR). Categorical variables were presented as absolute (n) and relative frequency (%). Chi-square or, when necessary, Fisher exact test, were applied to explore the association between categorical variables. Linear regression analysis and Spearman's correlations were used to assess the association between BACE1, other analytes and MS clinical characteristics. Difference in continuous variables between RR-MS patients and controls was evaluated using nonparametric Mann–Whitney test. A p value $\leq 0.05$ was considered statistically significant. When exploring the impact of BACE1 on CSF cytokine profile, considering the high number of variables, we corrected for multiple comparisons using Benjamini-Hochberg (B-H) procedure.

## 3. Results

### 3.1. Association of BACE1 CSF levels and MS clinical characteristics at the time of LP

The clinical and demographic characteristics of RR-MS patients are shown in Table 1.

**Table 1**
Clinical characteristics of MS and control patients.

| | | RR-MS patients n = 50 | Control patients n = 25 | |
|---|---|---|---|---|
| Sex, F | N (%) | 33 (66) | 11 (44) | p = 0.58 |
| Age, years | Mean, (SD) | 37 (11.5) | 48.99 (14.7) | p = 0.01* |
| Disease duration, months | Median, (IQR) | 4.9 (1.2 - 34.8) | - | |
| OCB presence, yes | N (%) | 40 (80) | - | |
| Radiological Activity | N (%) | 22 (44) | - | |
| EDSS at LP | Median, (IQR) | 1.5 (1 - 2) | - | |

Table 1 legend. Demographic and clinical characteristics of RR-MS and patients. Data are given as number (%) for dichotomic variables, and as median (interquartile ranges) for continuous variables.

* denotes statistical significance ($p < 0.05$) using a nonparametric Mann–Whitney test for continuous variables and Chi-square for categorial variables. Abbreviations: female (F), relapsing-remitting multiple sclerosis (RR-MS), oligoclonal bands (OCB), lumbar puncture (LP), expanded disability status scale (EDSS), interquartile range (IQR).

No significant associations were found between the CSF levels of BACE1 and age at LP and sex, in both the MS (age: Spearman's r = 0.231, p = 0.107, n = 50; sex: p = 0.48) and the control group (age: Spearman's r = 0.003, p = 0.988, n = 25; sex: p = 0.099).

In the MS group a significant correlation was found between BACE1 CSF concentrations and BREMS at onset (Spearman's r = 0.496, p = 0.0003, n = 48). No significant associations were observed with other clinical variables explored: disease duration (Spearman's r = 0.077, p = 0.596, n = 50), OCB (p = 0.574), EDSS at LP (Spearman's r = 0.167, p = 0.246, n = 50), and radiological activity (p = 0.74).

Exploring the association of BACE1 and neuropsychiatric and neuropsychological data collected at time of LP, we found a significant correlation with BDI-II scores (Spearman's r = 0.335, p = 0.028, n = 43). No significant correlations were found with STAI-Y state (Spearman's r = 0.202, p = 0.193; n = 43) and trait scores (Spearman's r = 0.158, p = 0.313, n = 43), and no significant correlations emerged with fatigue measured using the FSS (Spearman's r = 0.153, p = 0.321, n = 42). Notably, also comparing depressed and non-depressed patients, a significant difference was found in BACE1 CSF levels (Depressed patients median [IQR] = 6 [3-9.25]; Non-depressed, median [IQR] = 13 [16.5-24]; p = 0.0056) (Fig. 2). Finally, a significant negative correlation was found between BACE1 CSF levels and BVMT-R (Spearman's r = -0.385, p = 0.012, n = 42).

To explore the association between BACE1 CSF expression and prospective MS course, we evaluated the correlation between the CSF levels of this molecule at the time of diagnosis and the EDSS score evaluated after 3 years of follow-up. A significant positive correlation was evidenced between BACE1 CSF concentrations and EDSS at 3 years (Spearman's r = 0.467, p = 0.002, n = 41). Logistic regression confirmed a significant association between BACE1 and EDSS after 3 years of follow up (beta = 0.397, 95%CI 0-0.001, p = 0.008) also considering the effect of other possible confounders (age, sex, radiological activity, disease duration, OCB presence, DMTs).

### *3.2. Association between BACE1 and CSF biomarkers of neurodegeneration and inflammation*

To investigate the role of BACE1 in inflammatory and degenerative processes in MS, we first explored the associations between BACE1 and CSF biomarkers of neurodegeneration. Although no significant correlations were observed with amyloid β 1-40 (Spearman's r = -0.167, p = 0.247, n = 50), and amyloid β 1-42 (Spearman's r = 0.249, p = 0.082, n = 50), we found a significant positive correlation between BACE1 CSF concentrations and the amyloid β 42/40 ratio (Spearman's r = 0.334, p = 0.018, n = 50). Moreover, a significant correlation was found between BACE1 CSF levels and pTau concentrations (Spearman's r = 0.304, p = 0.032, n = 50). Finally, we found a significant positive correlation between BACE1 levels and lactate CSF concentrations (Spearman's r = 0.361, p = 0.01, n = 50).

We then analyzed the association between BACE1 and the CSF levels of a set of CSF inflammatory molecules in a subgroup of 40 RR-MS patients. Correlations were evidenced between BACE1 and different inflammatory cytokines, including IL-1β (Spearman's r = 0.341, p = 0.032, n = 40), IL-4 (Spearman's r = 0.488, p = 0.001, n = 40), IL-7 (Spearman's r = 0.359, p = 0.023, n = 40), IL-13 (Spearman's r = 0.396, p = 0.011, n = 40), IL-17 (Spearman's r = 0.511, p = 0.001, n = 40), G-CSF (Spearman's r = 0.517, p = 0.001, n = 40), IFN-γ (Spearman's r = -0.413, p = 0.008, n = 40), MCP-1 (Spearman's r = -0.323, p = 0.042, n = 40), and IL-9 (Spearman's r = 0.394, p = 0.012, n = 40). However, after correcting for multiple comparisons only the correlation with IL-4 (B-H corrected p = 0.01) and IL-17 (B-H corrected p = 0.01), IL-13 (B-H corrected p = 0.04), IL-9 (B-H corrected p = 0.04), and IFNγ (B-H corrected p = 0.04) were confirmed.

Fig. 1. BACE1 CSF levels, clinical and neuropsychological characteristics.

Fig. 2. BACE1 CSF levels and depression.

Fig. 3. BACE1 CSF levels, amyloid β 42/40 ratio, pTau and, CSF lactate.

Fig. 4. BACE1 CSF levels and inflammatory cytokines.

## 4. Discussion

BACE1 is a type I transmembrane aspartyl protease widely expressed in the brain, and particularly in neurons, oligodendrocytes, and astrocytes (Hampel et al., 2021). It is critically involved in the amyloidogenic pathway, producing an initial cleavage of the amyloid precursor protein (APP), which is then further cleaved by γ-secretases into 40 or 42 amino-acid long Aβ peptides (Hampel et al., 2021; Reiss et al., 2018). These Aβ peptides participate in the formation and regulation of amyloid plaques, forming dimers, oligomers, or insoluble amyloid fibrils critically involved in the pathogenesis of AD (Stertz et al., 2018). BACE1, by regulating Aβ production, may significantly impact neuronal degeneration, influencing different physiological process including neuronal and glial functioning (Hampel et al., 2021). Accordingly, Increased BACE1 CSF expression has been proposed as a prognostic marker, associated with higher risk of developing AD and with worse disease course (Mouton-Liger et al., 2020).

Here we explored the association between BACE1 and clinical characteristics of MS. In particular, we explored whether BACE1 CSF expression was correlated with important disease mechanisms such as amyloid homeostasis, oxidative stress and inflammation.

No significant differences were observed in BACE1 CSF concentrations between RR-MS patients at the time of diagnosis and patients with non-inflammatory and non-degenerative disorders including vascular leukoencephalopathy, polyneuropathies, normal pressure hydrocephalus, pseudotumor cerebri, functional neurological disorder, migraine, and spastic paraparesis. In addition, in both groups, BACE1 CSF levels were not associated with sex and age at LP. In MS patients, a significant association was found between increased expression of BACE1 and the presence of depression at the time of diagnosis measured with the BDI-II scale. Furthermore, a negative correlation was observed with visual-spatial memory performance evaluated by BVMT-R, suggesting that increased CSF BACE1 levels may be associated with increased mood and cognitive disturbances in RR-MS patients at the time of diagnosis.

Mood and cognitive alterations have been consistently reported in RR-MS already in the early stages of the disease (Binzer et al., 2019). It has been previously evidenced that inflammation may have a specific role in the pathogenesis of mood and cognitive disturbances in MS. Accordingly, higher levels of depression and anxiety at the time of

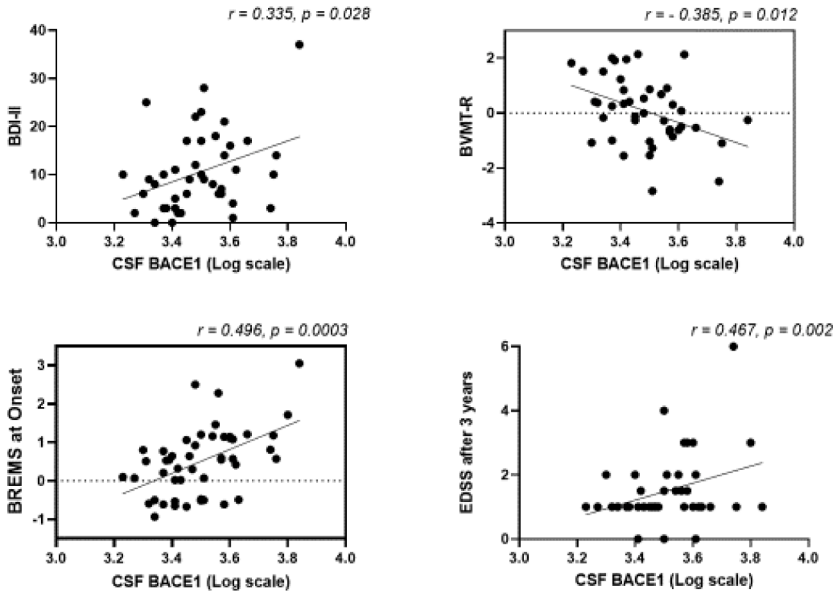


**Fig. 1.** Correlations between CSF levels of BACE1 and BDI-II, BVMT-R, BREMS at Onset, and EDSS after 3 years. Spearman's r and p-value are shown. Abbreviations: Beck Depression Inventory (BDI), cerebrospinal fluid (CSF), β-site amyloid precursor protein cleaving enzyme 1 (BACE1), Brief Visuospatial Memory Test – Revised (BVMT-R), Bayesian risk estimate for multiple sclerosis (BREMS), Expanded Disability Status Scale (EDSS).

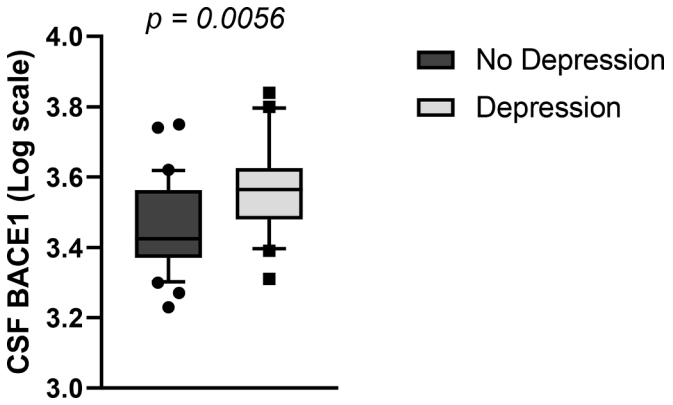


**Fig. 2.** Mann-Whitney test (M-W) was used to compare BACE1 levels in depressed and not-depressed RR-MS patients. Abbreviations: cerebrospinal fluid (CSF), β-site amyloid precursor protein cleaving enzyme 1 (BACE1).

diagnosis, have been associated with higher expression of inflammatory molecules in the CSF and related to higher risk of prospective disease activity in patients with MS (Mori et al., 2011). In addition, the presence of cognitive deficits at the time of diagnosis has been associated with long-term disability (Binzer et al., 2019; Kalb et al., 2018). These data suggest that mood and cognitive alterations may predispose MS patient to higher risk of neurodegeneration and disability progression.

In the present study, a positive correlation was found between BACE1 CSF levels and BREMS at onset, suggesting increased risk of progression in patients with higher levels of BACE1 at the time of diagnosis. In line with a proposed role of this molecule in promoting worse disease course, we found a positive correlation between the CSF concentration of BACE1 at the time of diagnosis and disability measured with the EDSS score after three years of follow-up.

These data suggest that BACE1 may be involved in mood and cognitive symptoms and may play a role in MS progression. Altered Aβ metabolism could be involved in both cognitive disfunction and disease progression in MS and may represent an important pathophysiological mechanism linking together inflammation and neurodegeneration in MS and in other neurological diseases (Hampel et al., 2021; Stampanoni Bassi et al., 2017; Stertz et al., 2018).

We explored the association between CSF BACE1 concentrations at the time of RR-MS diagnosis, and the levels of specific biomarker involved in neurodegeneration (Reiss et al., 2018). A significant positive correlation was observed between BACE1 and both CSF Aβ 1-42/1-40 ratio, a biomarker of Aβ pathology burden (Baiardi et al., 2018), and the levels of pTau, suggesting that BACE1 may be associated with increased amyloid synthesis and neuronal damage.

Notably, the contribution of neurodegeneration biomarkers in MS pathophysiology is still unclear. Reduced CSF Aβ 1-42 levels have been associated with increased disability (Pietroboni et al., 2020, 2019) and cognitive dysfunction (Mori et al., 2011), and may predict disability progression and global gray matter loss (Pietroboni et al., 2020, 2019). However, a recent study in RR-MS patients failed to find significant correlations between baseline amyloid levels and 3-year disability (Petitfour et al., 2022).

Both tau protein and lactate CSF concentrations may represent important biomarkers of neurodegeneration in MS being associated with increased axonal damage, mitochondrial dysfunction and oxidative stress (Barcelos et al., 2019; Szabo et al., 2020). The correlation between BACE1 CSF levels and pTau further supports a role of this molecule in neurodegeneration in MS and is in line with previous studies in AD patients showing a positive correlation between BACE1 and both tau and pTau levels (Timmers et al., 2017). Notably, a correlation between baseline CSF tau levels and early disability has been recently reported in MS (Virgilio et al., 2022). In addition, we found a positive correlation between BACE1 and CSF concentrations of lactate, suggesting that increased CSF BACE1 expression may be associated with enhanced oxidative stress and mitochondrial dysfunction (Albanese et al., 2016; Barcelos et al., 2019; Szabo et al., 2020). Increased lactate CSF levels have been associated with disability and risk of progression in RR-MS patients at the time of diagnosis (Albanese et al., 2016). Importantly, it has been shown that BACE1 overexpression reduced mitochondrial glucose oxidation in a human neuronal cell line (Findlay et al., 2015). Overall, these data indicate a role of BACE1 in both neurodegeneration and oxidative stress in MS.

BACE1 is involved in a number of physiological processes of neurons and glial cells (Guix et al., 2019). A role of BACE1 in central myelination has been reported, accordingly, BACE1 knockout mice presented lower myelination and reduced activation of neuregulin-1, a protein

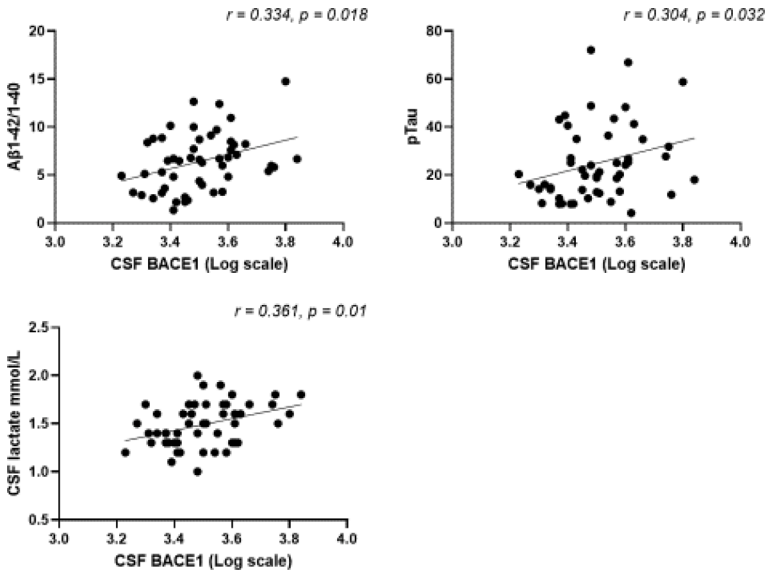


**Fig. 3.** Correlations between CSF levels of BACE1 and amyloid β 42/40 ratio, pTau and, CSF lactate. Spearman's r and p-value are shown. Abbreviations: cerebrospinal fluid (CSF), β-site amyloid precursor protein cleaving enzyme 1 (BACE1), amyloid β (Aβ), phosphorylated tau (pTau).

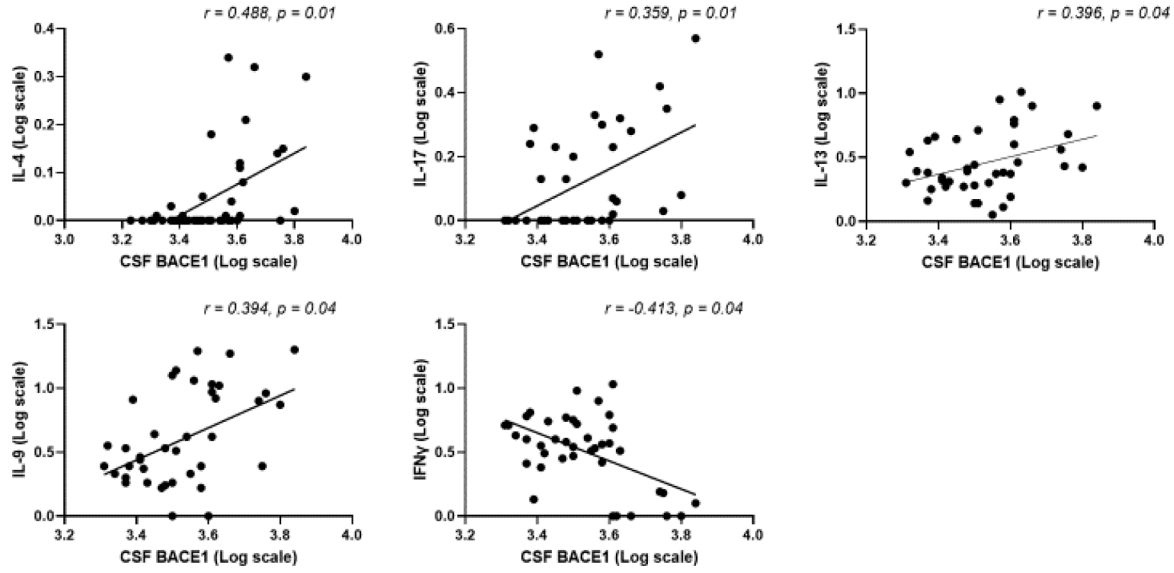


**Fig. 4.** Correlation analysis between CSF levels of BACE1 and pro-inflammatory and anti-inflammatory cytokines. Spearman's r and p-value after Benjamini-Hochberg (B-H) correction are shown. Abbreviations: cerebrospinal fluid (CSF), relapsing-remitting multiple sclerosis (RR-MS), β-site amyloid precursor protein cleaving enzyme 1 (BACE1), interleukin (IL), interferon (IFN).

implicated in the maintenance of the mature myelin sheath in oligodendrocytes (Hu et al., 2006). In addition, reduced CSF BACE1 activity has been reported in secondary progressive MS patients compared with RR-MS patients, suggesting progressive loss of remyelinating capacity in the late stages of disease (Mattsson et al., 2009). Recent research in AD evidenced an association between BACE1 CSF levels, CNS inflammation, and glial activation. In particular, abnormal activation of STAT3, a protein implicated in pro-inflammatory cytokine transcription, has been proposed as a transcriptional regulator of BACE1 (Millot et al., 2020). In addition, it has been shown that several major proinflammatory cytokines, such as TNF, IL-1, and IL-6, increased the IKK/IκB/NF-κB/BACE1 signaling pathway in mice (Kuhn et al., 2007; Zheng et al., 2020). Inhibition of the NF-κB pathway can reduce BACE1 activity, by influencing microglia and astrocyte activation and Aβ deposition in a murine model of AD (Qiao et al., 2021). Importantly, BACE1 is fundamental in experimental autoimmune encephalomyelitis (EAE) for naïve T-cells in T-helper17 differentiation, by regulating cAMP responses, calcium signaling, and PTEN levels (Hernandez-Mir et al., 2019). Notably, a recent study showed that BACE1 knockout mice did not develop EAE after induction (Hernandez-Mir et al., 2019).

Based on this evidence, we investigated the association between BACE1 and CSF inflammation in our group of RR-MS patients. We found a positive correlation between the CSF levels of BACE1 and those of different inflammatory and anti-inflammatory CSF cytokines, including IL-17, IL-13, IL-9, G-CSF and IL-4, and a negative correlation with IFN-γ.

It has been previously reported that IL-17 released by activated microglia may influence both BACE1 CSF expression and cognitive and mood symptoms in rats stimulated with lipopolysaccharide (Sun et al., 2015). Accordingly, administration of IL-17 inhibiting antibodies reduced BACE1 production and cognitive dysfunction (Sun et al., 2015). In our study we found a positive correlation between BACE1 and IL-9 CSF levels; however, a previous study showed that BACE1 inhibition

in mice was associated with increased levels of IL-9 (Stertz et al., 2018). In addition, we found a negative association between the CSF levels of BACE1 and IFN-γ, although a preclinical study reported an association between BACE1 and increased levels of IFN-γ (Zhao et al., 2011).

In line with the role of inflammation in altering Aβ metabolism, it has been reported that CSF levels of Aβ1–42 were negatively correlated with the number of Gd+ lesions at MRI (Mori et al., 2011), and reduced CSF Aβ1–42 levels were associated with cognitive deficits and impaired synaptic plasticity (Mori et al., 2011). In addition, in the CSF of RR-MS patients, Aβ1–42 concentrations were inversely correlated with the levels of different proinflammatory and anti-inflammatory cytokines, suggesting that inflammatory CSF milieu may influence Aβ expression (Stampanoni Bassi et al., 2017). Our data may suggest that CSF expression of BACE1 is significantly influenced by the levels of central inflammation in MS, proposing BACE1 as an important player able to modulate the effects of inflammation on Aβ brain homeostasis.

In the present study, we showed for the first time that increased CSF BACE1 expression may predict worse disease course of MS influencing inflammatory and neurodegenerative processes.

However, our statistical analysis demonstrated a slight association between CSF BACE1 levels and inflammatory and neurodegenerative processes. Studies conducted on larger populations are needed to better elucidate the role of BACE1 in MS pathophysiology.

This study has important limitations, including the low number of participants and the lack of MRI measures of neurodegeneration. Moreover, we included RR-MS patients with short disease duration, it is therefore important to explore the role of BACE1 in patients with longer disease duration and with progressive MS phenotypes. Further studies are required to better clarify the role of BACE1 in MS progression and its relevance as a disease biomarker.

In conclusion, BACE1 has a limited relevance in the mechanisms of neurodegeneration, oxidative stress and inflammation in MS, influencing mood, cognitive disorders and disability progression.

## Author Contributions

Conceptualization, A.B., D.C. and M.S.B.; writing—original draft preparation, A.B. and M.S.B.; writing—review and editing, A.B., E.I., D. C., M.S.B.; data collection/curation, E.D., F.A., L.G., G.G., A.Bo., R.F., A. F., F.C., F.DV., A.M., L.Gu., G.Mat.; funding acquisition, D.C., G.M.; statistical analysis, A.B., M.S.B. All authors have read and agreed to the published version of the manuscript.

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## Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Neuromed Research Institute (cod. 06-17).

## Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

## Data Availability Statement

Anonymized datasets are available upon reasonable request to the corresponding author.

## Declaration of Competing Interest

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: F. B. (Fabio Buttari) acted as Advisory Board members of Teva and Roche and received honoraria for speaking or consultation fees from Merck Serono, Teva, Biogen Idec, Sanofi, and Novartis and non-financial support from Merck Serono, Teva, Biogen Idec, and Sanofi. R.F. received honoraria for serving on scientific advisory boards or as a speaker from Biogen, Novartis, Roche, and Merck and funding for research from Merck. D.C. (Diego Centonze) is an Advisory Board member of Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva and received honoraria for speaking or consultation fees from Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Gen-zyme, and Teva. He is also the principal investigator in clinical trials for Bayer Schering, Biogen, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, and Teva. His preclinical and clinical research was supported by grants from Bayer Schering, Biogen Idec, Celgene, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva. G.M. (Giuseppe Matarese) reports receiving research grant support from Merck, Biogen, and Novartis and advisory board fees from Merck, Biogen, Novartis, and Roche. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. A.B., E.D., F.A., L. G., E.I., G.G., A.Bo., R.F., A.F., F.C., F.DV., A.Mu., L.Gu., G.M, M.S.B.: nothing to report.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2023.104528.

## References

Albanese, M., Zagaglia, S., Landi, D., Boffa, L., Nicoletti, C.G., Marciani, M.G., Mandolesi, G., Marfia, G.A., Buttari, F., Mori, F., Centonze, D., 2016. Cerebrospinal fluid lactate is associated with multiple sclerosis disease progression. J. Neuroinflammation 13, 36. https://doi.org/10.1186/s12974-016-0502-1.

Baiardi, S., Abu-Rumeileh, S., Rossi, M., Zenesini, C., Bartoletti-Stella, A., Polischi, B., Capellari, S., Parchi, P., 2018. Antemortem CSF A β 42/A β 40 ratio predicts Alzheimer's disease pathology better than A β 42 in rapidly progressive dementias. Ann. Clin. Transl. Neurol. acn3. 697 https://doi.org/10.1002/acn3.697.

Barcelos, I.P.de, Troxell, R.M., Graves, J.S., 2019. Mitochondrial Dysfunction and Multiple Sclerosis. Biology (Basel) 8, 37. https://doi.org/10.3390/biology8020037.

Bergamaschi, R., Montomoli, C., Mallucci, G., Lugaresi, A., Izquierdo, G., Grand'Maison, F., Duquette, P., Shaygannejad, V., Alroughani, R., Grammond, P., Boz, C., Iuliano, G., Zwanikken, C., Petersen, T., Lechner-Scott, J., Hupperts, R., Butzkueven, H., Pucci, E., Oreja-Guevara, C., Cristiano, E., Pia Amato, M.P., Havrdova, E., Fernandez-Bolanos, R., Spelman, T., Trojano, M., 2015. BREMSO: a simple score to predict early the natural course of multiple sclerosis. Eur. J. Neurol. 22, 981–989. https://doi.org/10.1111/ene.12696.

Binzer, S., McKay, K.A., Brenner, P., Hillert, J., Manouchehrinia, A., 2019. Disability worsening among persons with multiple sclerosis and depression. Neurology 93, e2216–e2223. https://doi.org/10.1212/WNL.0000000000008617.

Cole, S.L., Vassar, R., 2007. The Alzheimer's disease Beta-secretase enzyme, BACE1. Mol. Neurodegener. 2, 22. https://doi.org/10.1186/1750-1326-2-22.

Diker, S., Has, A.C., Kurne, A., Göçmen, R., Oğuz, K.K., Karabudak, R., 2016. The association of cognitive impairment with gray matter atrophy and cortical lesion load in clinically isolated syndrome. Mult. Scler. Relat. Disord. 10, 14–21. https://doi.org/10.1016/j.msard.2016.08.008.

Eshaghi, A., Marinescu, R.V, Young, A.L., Firth, N.C., Prados, F., Jorge Cardoso, M., Tur, C., De Angelis, F., Cawley, N., Brownlee, W.J., De Stefano, N., Laura Stromillo, M., Battaglini, M., Ruggieri, S., Gasperini, C., Filippi, M., Rocca, M.A., Rovira, A., Sastre-Garriga, J., Geurts, J.J.G., Vrenken, H., Wottschel, V., Leurs, C.E., Uitdehaag, B., Pirpamer, L., Enzinger, C., Ourselin, S., Gandini Wheeler-Kingshott, C.

A., Chard, D., Thompson, A.J., Barkhof, F., Alexander, D.C., Ciccarelli, O., 2018. Progression of regional grey matter atrophy in multiple sclerosis. Brain 141, 1665–1677. https://doi.org/10.1093/brain/awy088.

Ewers, M., Cheng, X., Zhong, Z., Nural, H.F., Walsh, C., Meindl, T., Teipel, S.J., Buerger, K., He, P., Shen, Y., Hampel, H., 2011. Increased CSF-BACE1 activity associated with decreased hippocampus volume in Alzheimer's Disease. J. Alzheimer's Dis. 25, 373–381. https://doi.org/10.3233/JAD-2011-091153.

Findlay, J.A., Hamilton, D.L., Ashford, M.L.J., 2015. BACE1 activity impairs neuronal glucose oxidation: rescue by beta-hydroxybutyrate and lipoic acid. Front. Cell. Neurosci. 9 https://doi.org/10.3389/fncel.2015.00382.

Guix, F.X., Sartório, C.L., ILL-Raga, G., 2019. BACE1 translation: at the crossroads between Alzheimer's Disease neurodegeneration and memory consolidation. J. Alzheimer's Dis. Reports 3, 113–148. https://doi.org/10.3233/ADR-180089.

Hampel, H., Vassar, R., De Strooper, B., Hardy, J., Willem, M., Singh, N., Zhou, J., Yan, R., Vanmechelen, E., De Vos, A., Nisticò, R., Corbo, M., Imbimbo, B., Pietro, Streffer, J., Voytyuk, I., Timmers, M., Tahami Monfared, A.A., Irizarry, M., Albala, B., Koyama, A., Watanabe, N., Kimura, T., Yarenis, L., Lista, S., Kramer, L., Vergallo, A., 2021. The β-Secretase BACE1 in Alzheimer's Disease. Biol. Psychiatry 89, 745–756. https://doi.org/10.1016/j.biopsych.2020.02.001.

Hernandez-Mir, G., Raphael, I., Revu, S., Poholek, C.H., Avery, L., Hawse, W.F., Kane, L. P., McGeachy, M.J., 2019. The Alzheimer's Disease–Associated Protein BACE1 Modulates T Cell Activation and Th17 Function. J. Immunol. 203, 665–675. https://doi.org/10.4049/jimmunol.1800363.

Hu, X., Hicks, C.W., He, W., Wong, P., Macklin, W.B., Trapp, B.D., Yan, R., 2006. Bace1 modulates myelination in the central and peripheral nervous system. Nat. Neurosci. 9, 1520–1525. https://doi.org/10.1038/nn1797.

Kalb, R., Beier, M., Benedict, R.H., Charvet, L., Costello, K., Feinstein, A., Gingold, J., Goverover, Y., Halper, J., Harris, C., Kostich, L., Krupp, L., Lathi, E., LaRocca, N., Thrower, B., DeLuca, J., 2018. Recommendations for cognitive screening and management in multiple sclerosis care. Mult. Scler. J. 24, 1665–1680. https://doi.org/10.1177/1352458518803785.

Krupp, L.B., 1989. The Fatigue Severity Scale. Arch. Neurol. 46, 1121. https://doi.org/10.1001/archneur.1989.00520460115022.

Kuhn, P.-H., Marjaux, E., Imhof, A., De Strooper, B., Haass, C., Lichtenthaler, S.F., 2007. Regulated Intramembrane Proteolysis of the Interleukin-1 Receptor II by α-, β-, and γ-Secretase. J. Biol. Chem. 282, 11982–11995. https://doi.org/10.1074/jbc.M700356200.

Mattsson, N., Axelsson, M., Haghighi, S., Malmeström, C., Wu, G., Anckarsäter, R., Sankaranarayanan, S., Andreasson, U., Fredrikson, S., Gundersen, A., Johnsen, L., Fladby, T., Tarkowski, A., Trysberg, E., Wallin, A., Anckarsäter, H., Lycke, J., Andersen, O., Simon, A., Blennow, K., Zetterberg, H., 2009. Reduced cerebrospinal fluid BACE1 activity in multiple sclerosis. Mult. Scler. J. 15, 448–454. https://doi.org/10.1177/1352458508100031.

Millot, P., San, C., Bennana, E., Porte, B., Vignal, N., Hugon, J., Paquet, C., Hosten, B., Mouton-Liger, F., 2020. STAT3 inhibition protects against neuroinflammation and BACE1 upregulation induced by systemic inflammation. Immunol. Lett. 228, 129–134. https://doi.org/10.1016/j.imlet.2020.10.004.

Mori, F., Rossi, S., Sancesario, G., Codecà, C., Mataluni, G., Monteleone, F., Buttari, F., Kusayanagi, H., Castelli, M., Motta, C., Studer, V., Bernardi, G., Koch, G., Bernardini, S., Centonze, D., 2011. Cognitive and cortical plasticity deficits correlate with altered amyloid-β CSF levels in multiple sclerosis. Neuropsychopharmacology 36, 559–568. https://doi.org/10.1038/npp.2010.187.

Mouton-Liger, F., Dumurgier, J., Cognat, E., Hourregue, C., Zetterberg, H., Vanderstichele, H., Vanmechelen, E., Bouaziz-Amar, E., Blennow, K., Hugon, J., Paquet, C., 2020. CSF levels of the BACE1 substrate NRG1 correlate with cognition in Alzheimer's disease. Alzheimers. Res. Ther. 12, 88. https://doi.org/10.1186/s13195-020-00655-w.

Pedrabissi, L., & Santinello, M., 1989. Verifica della validità dello STAI forma Y di Spielberger [Verification of the validity of the STAI, Form Y, by Spielberger].

Petitfour, J., Ayrignac, X., Ginestet, N., Prin, P., Carra-Dallière, C., Hirtz, C., Charif, M., Lehmann, S., Labauge, P., 2022. CSF β-amyloid is not a prognostic marker in multiple sclerosis patients. Mult. Scler. Relat. Disord. 68, 104096 https://doi.org/10.1016/j.msard.2022.104096.

Pietroboni, A.M., Caprioli, M., Carandini, T., Scarioni, M., Ghezzi, L., Arighi, A., Cioffi, S., Cinnante, C., Fenoglio, C., Oldoni, E., De Riz, M.A., Basilico, P., Fumagalli, G.G., Colombi, A., Giulietti, G., Serra, L., Triulzi, F., Bozzali, M., Scarpini, E., Galimberti, D., 2019. CSF β-amyloid predicts prognosis in patients with multiple sclerosis. Mult. Scler. J. 25, 1223–1231. https://doi.org/10.1177/1352458518791709.

Pietroboni, A.M., Colombi, A., Carandini, T., Contarino, V.E., Ghezzi, L., Fumagalli, G.G., Arighi, A., Fenoglio, C., De Riz, M.A., Triulzi, F., Scarpini, E., Galimberti, D., 2020. Low CSF β-amyloid levels predict early regional grey matter atrophy in multiple sclerosis. Mult. Scler. Relat. Disord. 39, 101899 https://doi.org/10.1016/j.msard.2019.101899.

Pravatà, E., Rocca, M.A., Valsasina, P., Riccitelli, G.C., Gobbi, C., Comi, G., Falini, A., Filippi, M., 2017. Gray matter trophism, cognitive impairment, and depression in patients with multiple sclerosis. Mult. Scler. J. 23, 1864–1874. https://doi.org/10.1177/1352458517692886.

Qiao, A., Li, J., Hu, Y., Wang, J., Zhao, Z., 2021. Reduction BACE1 expression via suppressing NF-κB mediated signaling by Tamibarotene in a mouse model of Alzheimer's disease. IBRO Neurosci. Reports 10, 153–160. https://doi.org/10.1016/j.ibneur.2021.02.004.

Reiss, A.B., Arain, H.A., Stecker, M.M., Siegart, N.M., Kasselman, L.J., 2018. Amyloid toxicity in Alzheimer's disease. Rev. Neurosci. 29, 613–627. https://doi.org/10.1515/revneuro-2017-0063.

Sica, C., & Ghisi, M. (Nova S.P., 2007. The Italian versions of the Beck anxiety inventory and the beck depression inventory-II: psychometric properties and discriminant power.

Stampanoni Bassi, M., Garofalo, S., Marfia, G.A., Gilio, L., Simonelli, I., Finardi, A., Furlan, R., Sancesario, G.M., Di Giandomenico, J., Storto, M., Mori, F., Centonze, D., Iezzi, E., 2017. Amyloid-β homeostasis bridges inflammation, synaptic plasticity deficits and cognitive dysfunction in multiple sclerosis. Front. Mol. Neurosci. 10 https://doi.org/10.3389/fnmol.2017.00390.

Stertz, L., Contreras-Shannon, V., Monroy-Jaramillo, N., Sun, J., Walss-Bass, C., 2018. BACE1-deficient mice exhibit alterations in immune system pathways. Mol. Neurobiol. 55, 709–717. https://doi.org/10.1007/s12035-016-0341-1.

Sun, J., Zhang, S., Zhang, Xiang, Zhang, Xiaobao, Dong, H., Qian, Y., 2015. IL-17A is implicated in lipopolysaccharide-induced neuroinflammation and cognitive impairment in aged rats via microglial activation. J. Neuroinflammation 12, 165. https://doi.org/10.1186/s12974-015-0394-5.

Szabo, L., Eckert, A., Grimm, A., 2020. Insights into disease-associated tau impact on mitochondria. Int. J. Mol. Sci. 21, 6344. https://doi.org/10.3390/ijms21176344.

The Goldman Consensus statement on depression in multiple sclerosis, 2005. Mult. Scler. J. 11, 328–337. https://doi.org/10.1191/1352458505ms1162oa.

Timmers, M., Barão, S., Van Broeck, B., Tesseur, I., Slemmon, J., De Waepenaert, K., Bogert, J., Shaw, L.M., Engelborghs, S., Moechars, D., Mercken, M., Van Nueten, L., Tritsmans, L., de Strooper, B., Streffer, J.R., 2017. BACE1 Dynamics Upon Inhibition with a BACE Inhibitor and Correlation to Downstream Alzheimer's Disease Markers in Elderly Healthy Participants. J. Alzheimer's Dis. 56, 1437–1449. https://doi.org/10.3233/JAD-160829.

Virgilio, E., Vecchio, D., Crespi, I., Puricelli, C., Barbero, P., Galli, G., Cantello, R., Dianzani, U., Comi, C., 2022. Cerebrospinal fluid biomarkers and cognitive functions at multiple sclerosis diagnosis. J. Neurol. 269, 3249–3257. https://doi.org/10.1007/s00415-021-10945-4.

Zhao, J., O'Connor, T., Vassar, R., 2011. The contribution of activated astrocytes to Aβ production: Implications for Alzheimer's disease pathogenesis. J. Neuroinflammation 8, 150. https://doi.org/10.1186/1742-2094-8-150.

Zheng, Y., Deng, Y., Gao, J., Lv, C., Lang, L., Shi, J., Yu, C., Gong, Q., 2020. Icariside II inhibits lipopolysaccharide-induced inflammation and amyloid production in rat astrocytes by regulating IKK/IκB/NF-κB/BACE1 signaling pathway. Acta Pharmacol. Sin. 41, 154–162. https://doi.org/10.1038/s41401-019-0300-2.

Zhong, Z., Ewers, M., Teipel, S., Bürger, K., Wallin, A., Blennow, K., He, P., McAllister, C., Hampel, H., Shen, Y., 2007. Levels of β-secretase (BACE1) in cerebrospinal fluid as a predictor of risk in mild cognitive impairment. Arch. Gen. Psychiatry 64, 718. https://doi.org/10.1001/archpsyc.64.6.718.