Association of CSF sTREM2, a marker of microglia activation, with cholinergic basal forebrain volume in major depressive disorder

Running Title: Cholinergic integrity and microglia response in MDD

Abstract

Background: Inflammatory mechanisms are believed to contribute to the manifestation of major depressive disorder (MDD). Central cholinergic activity may moderate this effect. Here, we tested if volume of the cholinergic basal forebrain is associated with cerebrospinal fluid (CSF) levels of sTREM2 as a marker of microglial activation in people with late life MDD.

Methods: Basal forebrain volume was determined from structural MRI scans and levels of CSF sTREM2 with immunoassay in 29 people with late-life MDD and 20 healthy older controls at baseline and 3 years follow-up. Associations were determined using Bayesian analysis of covariance.

Results: We found moderate level of evidence for an association of lower CSF levels of sTREM2 at 3 years follow up with MDD (Bayes factor in favor of an effect = 7.9). This level of evidence prevailed when controlling for overall antidepressant treatment and CSF levels of markers of AD pathology, *i.e.*, Aβ42/Aβ40, $ptau_{181}$ and total tau. Evidence was in favour of absence of an effect for baseline levels of CSF sTREM2 in MDD cases and for baseline and follow up data in controls.

Limitations: The sample size of repeated CSF examinations was relatively small. Therefore, we used Bayesian sequential analysis to assess if effects were affected by sample size. Still, the number of cases was too small to stratify effects for different antidepressive treatments.

Conclusions: Our data agree with the assumption that central cholinergic system integrity may contribute to regulation of microglia activity in late-life MDD.

Keywords: Microglia activation; basal forebrain; late life major depressive disorder; cerebrospinal fluid; MRI

Introduction

Dysregulation of monoamine transmitter function is the best established pathogenetic factor of major depressive disorder (MDD) (Blokhin et al., 2020). However, the long temporal delay between the restoration of monoamine levels at the synapse level and the clinical effect of antidepressive drugs, and the wide range of polygenetic risk factors (Hyman, 2014), point to alternative or complementary pathways upstream of neurotransmitter disturbances. One of these are inflammatory mechanisms, as indicated by higher risk of MDD following severe infections (Benros et al., 2013) and higher levels of pro-inflammatory cytokines in plasma in people with MDD as compared with controls (Muller, 2014). Parts of this effect may be mediated by microglial activation (Menard et al., 2016). Consistently, a study using PET found increased levels of translocator protein density as marker of microglial activation in the brains of people with a major depressive episode due to MDD compared with controls (Setiawan et al., 2015).

The basal forebrain cholinergic system of the brain was found to moderate the inflammatory response to different pathologies (Shytle et al., 2004). For example, selective stimulation or suppression of basal forebrain cholinergic neuron activity in mice affected the peripheral expression of tumor necrosis factor (TNF) levels in response to endotoxemia (Lehner et al., 2019). This finding suggests that central action of acetylcholine receptors in the brain may suppress circulating TNF and other proinflammatory cytokines. Similarly, the centrally acting acetylcholinesterase inhibitor galantamine has been found to suppress acid aspiration-induced acute respiratory syndrome in rabbits (Yang et al., 2018).

Based on the possible role of cholinergic integrity in inflammation and possibly altered microglia activation in MDD, we tested the hypothesis that the volume of the cholinergic basal forebrain as measured by MRI (Kilimann et al., 2014) was associated with CSF levels of soluble Triggering Receptor Expressed on Myeloid Cells 2 (sTREM2), a biomarker of microglial activation (Konishi and Kiyama, 2020). We carried out this test in people diagnosed with MDD, at baseline and after 3 years of follow up. For comparison, we also studied these associations in clinically healthy controls.

Material and Methods

Study sample

A total of 133 participants took part in a three-year longitudinal study, which was approved by the Nathan Kline Institute for Psychiatric Research (NKI) and New York University School of Medicine (NYU SoM) institutional review boards. Of the 133 participants, 51 participants completed an optional lumbar puncture (LP) at baseline and 39 participants completed the three-year follow-up LP. We used the baseline and three years follow-up data of 31 cases with a clinical diagnosis of MDD according to DSM-IV criteria (American Psychiatric Association, 1994). Results were compared with 20 elderly healthy controls. We excluded cases without an available CSF sample, leaving 29 MDD cases and 20 controls. Basic demographics are given in Table 1. All participants provided written informed consent. The study was conducted in accord with the Helsinki Declaration of 1975.

Antidepressive treatment

This was an observational study so that antidepressive treatment was not regualted. Distribution of antidepressive treatment over time points and MDD cases is shown in Table 1. The following drug classes were used for treatment: the selective serotonine reuptake inhibitors Sertraline, Fluoxetine, Escitalopram, and Paroxetine; the serotonin and noradrenaline reuptake inhibitors Venlafaxine and Duloxetine; the dopamine reuptake inhibitor and releaser Bupropion; Trazodone; the mood stabilizer Lamotrigine; Mirtazapine.

Study Description

A more detailed description for the study procedure and the follow-up visits can be found in (Pomara et al., 2021). In brief, participants completed four study visits over the course of three years (i.e., baseline, three-year follow-up). At each visit, participants had a clinical evaluation, including measures of depression severity (e.g., Hamilton Depression Rating Scale) and cognitive status (e.g., memory and executive function tests). Participants also had medical and psychiatric

evaluations by a board-certified psychiatrist. Blood draws for biomarker determination and routine laboratory tests were taken at each visit.

Biomarker Determination (CSF)

A fasting lumbar puncture was completed at the baseline and three-year follow-up visit. CSF was processed and analyzed for levels of amyloid beta 1-40 and 1-42 and tau and ptau proteins as described in (Pomara et al., 2012). CSF sTREM2 concentration was measured using an in-house immunoassay with electrochemiluminescence detection, as previously described in detail in (Banerjee et al., 2020).

MRI data acquisition and analysis

MRI scans were collected at the baseline from all participants. The acquisition was performed on a 1.5 T Siemens Vision system (Erlangen, Germany) at the Nathan S. Kline Institute for Psychiatric Research, NY, USA. Images were acquired using a sagittal magnetization prepared rapid gradient-echo sequence [MPRAGE; repetition time (TR)/echo time (TE)=11.4/11.9 ms, 1 excitation (NEX), matrix=256 x 256, FOV=307 mm, 1.2mm^3 isotropic voxel, 172 slices, no gap].

MRI data processing followed procedures described previously (Kilimann et al., 2014), implemented in SPM8 and the VBM8-toolbox in Matlab. The basal forebrain region was determined according to a map from an *in cranio* post mortem MRI scan and histology of a single individual's brain (Kilimann et al., 2014). The total intracranial volume (TIV) was used in the statistical model to account for differences in head size, and was calculated as the sum of the total segmented gray matter, white matter and cerebrospinal fluid volumes in native space.

Statistical analysis

Statistical analyses were conducted in a Bayesian framework to allow estimation of model plausibility and determining effect sizes with credibility intervals.

Demographic variables were compared between MDD and control groups using Bayesian contingency table test for sex and Bayesian independent t-tests for interval scaled variables.

Effects of cholinergic basal forebrain were evaluated in four independent Bayesian ANCOVA models, featuring controls and MDD patients, and baseline and follow-up sTREM2 levels, respectively. We determined effects of basal forebrain volumes on CSF TREM2 controlling for age, sex, and (in the MDD cases) cumulated antidepressive treatment. These index models were compared with the null models only containing the covariates. We used *Bayes factor (BF) hypothesis testing* to compare the index models containing the marker of interest against the null model. This approach allowed us to accept the best possible hypothesis for the data (Goodman, 2008; Wagenmakers et al., 2018).

For all analyses, we used *Jeffreys' Amazing Statistics Program (*JASP Version 0.11), available at jasp-stats.org. We report the Bayes Factor (BF_{10}) quantifying evidence against the null hypotheses. To address potential issues with non-normally distributed residuals in the multiple regressions we applied Markov-Monte Carlo chain sampling to each analysis 1,000 times. For the t-tests we used the JASP default Cauchy prior, for the ANCOVA models the JASP default JZS prior. We decided a priori to follow up the size of a marker effect only for index models with at least moderate plausibility, i.e., $BF_{10} > 3$. For these effects we used correlation analysis for effect size estimation and sequential analysis to determine the effects in dependence of the sample size. Additionally, we conducted a robustness check for different values of the JZS prior.

Results

Demographics

As shown in Table 1, evidence for a difference in sex distribution between MDD cases and controls was anecdotal, differences in age and basal forebrain volume were more likely to be absent. In respect to CSF levels of the Alzheimer's disease (AD) core biomarkers Aβ42/Aβ40 ratio, $ptau_{181}$ and t-tau, evidence was in favor of absence of a group difference. For baseline levels of CSF sTREM2 we found an anecdotal level of evidence for a decline in MDD compared with controls.

CSF sTREM2 levels and basal forebrain volume

In controls, we found anecdotal evidence for the absence of an effect of basal forebrain volume on CSF sTREM2 levels both at baseline and at three years follow-up (Table 2). In MDD cases we found anecdotal evidence for the absence of an effect on baseline levels of sTREM2 in CSF (Table 3a). When **additionally controlling for treatment** to this model, BF_{10} was 0.516, i.e., absence of an effect of basal forebrain volume on CSF-TREM2 levels at baseline was 1.94 times more likely than the presence of such effect.

For three year follow-up levels of CSF sTREM2, effects were moderately in favor of an effect of basal forebrain volume in the MDD cases (Table 3b). When **additionally controlling for treatment**, BF_{10} became 5.582, i.e., a moderate plausibility of the effect of basal forebrain volume was preserved. When we included **Aβ42/Aβ40 ratio** into the null model, we found moderate evidence in favor of an effect of basal forebrain volume (BF_{10} = 5.94), when we included **t-tau or p-tau 181**, BF_{10} became 2.84, providing anecdotal evidence for an effect of basal forebrain.

Assessing the treatment effect itself on CSF sTREM2 levels revealed anecdotal evidence for the absence of an effect of baseline treatment on baseline CSF sTREM2 levels, and of cumulated treatment across baseline and follow-up on CSF sTREM2 levels at three years follow-up.

The correlation between basal forebrain volume and CSF sTREM2 levels at three-years follow-up in the MDD cases is shown in Figure 1. The corresponding Pearson's correlation coefficient was -0.61 (95% credibility interval -0.81 to -0.22). The Bayes Factor robustness check showed that evidence in favor of H1 was robust across the entire range of prior specifications (Figure 2).

The sequential analysis showed that e**v**idence for H1 started stabilizing already after 16 cases had been included (Figure 3).

Discussion

We found that larger basal forebrain volumes at baseline were associated with lower levels of CSF sTREM2 at three years follow-up, but not at baseline examination, in people with MDD. Effects were inconclusive in the controls.

These data support the notion that inflammation may play a role in the pathogenesis of MDD and may be moderated by the integrity of the cholinergic basal forebrain. In a previous analysis of this population we had observed a positive association of acetylcholinesterase (AChE) activity, indicating reduced cholinergic tone, with CSF sTREM2 levels at baseline both in the MDD cases and the controls (Pomara et al., 2021). The observation of a negative association of basal forebrain volume with CSF sTREM2 levels in the current analysis agrees with these findings.

CSF sTREM2 levels have previously been studied in Alzheimer's disease (AD), with some studies reporting increases that were associated with mutations in the TREM2 gene (McGrowder et al., 2021; Piccio et al., 2016). In healthy older individuals CSF sTREM2 levels were associated with microglia activation as assessed by translocator protein (TSPO) binding using PET (Toppala et al., 2021). Increases of CSF sTREM in AD may occur as indicator of microglial response to amyloid aggregation (Ewers et al., 2020) leading to a reduction of amyloid accumulation rate and rate of clinical progression (Edwin et al., 2020). In contrast, our cohort of MDD cases exhibited numerically lower values of CSF sTREM2 levels at baseline and follow-up compared with controls, with weak evidence for a group difference. So far, CSF sTREM2 levels have not yet been reported in other MDD cohorts than ours.

Peripheral plasma markers were numerically increased in the MDD cases compared with controls, but there was not a general inflammatory status in the late life MDD cases of our cohort (Pomara et al., 2021). This agrees with an earlier report that among 23 plasma cytokines found only one that was increased in late life MDD compared with controls (Lee et al., 2009) whereas other studies found increased levels of plasma IL6 in late life MDD (Bremmer et al., 2008). Raison and Miller have pointed out that alterations of inflammatory markers in MDD are typically robustly below pathological levels. Therefore, they argue that these alterations do not render MDD an inflammatory disease but rather suggest that inflammatory mechanisms contribute to

the expression or maintenance of MDD symptomatology in some patients (Raison and Miller, 2013).

The negative association of basal forebrain at baseline with sTREM2 levels would agree with the assumption that central cholinergic activity may reduce microglial activation. In contrast to the effects of AChE in the previously reported study (Pomara et al., 2021) that may reflect an effect of cholinergic tone as a state marker, basal forebrain volume may represent a trait marker of cholinergic system integrity that is associated with lower levels of CSF sTREM2 at follow-up. Levels of CSF sTREM2 levels indicate microglia activity. Markers of neuroinflammation were found increased in some and decreased in other studies in late life depression (Hannestad et al., 2013; Su et al., 2016). Our data indicate that cholinergic function and structural integrity may be a possible factor that accounts for some of these differences.

We controlled the effects for influence of subclinical AD pathology using Aβ42/40, ptau and t-tau levels in CSF. Late life MDD is a risk factor for AD (Diniz et al., 2013) suggesting that subclinical AD may play a role in these cases. However, the effects of basal forebrain volume on levels of CSF sTREM2 were preserved even when taking Aβ and tau markers into account. It should be noted that our sample including the depressed cohort was cognitively unimpaired and had no indication of preclinical AD in CSF. Thus, these findings may not be pertinent to depressed individuals with some cognitive decline and potential preclinical or prodromal AD, where loss of forebrain volume may accentuate pro-inflammatory response to AD pathology.

Limitations

Since this is the first cohort to report sTREM2 levels in MDD cases, our data need independent confirmation. The lack of increase of peripheral inflammatory markers in MDD, including plasma IL6, is in contrast to several previous studies (Nobis et al., 2020) the majority of which, however, included younger cohorts. Another limitation of this study is the small sample size which is related to the difficulties of repeated CSF examinations in MDD cases and controls. We used sequential analysis in a Bayesian framework to assess if effects were affected by sample size. The effect stabilized at n = 16 cases, still, higher numbers would increase confidence in the

findings. We had controlled the effects for a possible confound by antidepressant treatment. We found no conclusive evidence for an effect of antidepressant treatment over three years on CSF sTREM2 levels. In addition, treatment did not substantially affect the association of CSF sTREM2 levels with basal forebrain volume. However, the small number of cases precluded controlling for different antidepressant mediations that may alter inflammatory markers differentially (Baumeister et al., 2016). Due to the small number of cases we could also not assess if the association between basal forebrain volume and CSF sTREM2 levels was moderated by the actual clinical syndrome of remitted, continuous or recurrent depression. Of note, cholinergic basal forebrain is a surrogate marker but not a direct assessment of the integrity of cholinergic input into the cerebrum (Teipel et al., 2020). Measures of cholinergic system functional integrity, such as nicotinic receptor binding (Tiepolt et al., 2021) or vesicular acetylcholine transporter (Giboureau et al., 2010) using molecular PET tracers, may provide further insight into the association of cholinergic activity and inflammatory response in future studies in MDD. A strength of our study is its longitudinal design, revealing effects of baseline basal forebrain structural integrity at follow-up.

In summary, we did not find an indication of a chronic inflammatory state in this late life MDD cohort, in contrast to several, but not all previous studies. CSF levels of sTREM2 as a marker of microglia response were associated with cholinergic basal forebrain volume at baseline suggesting that cholinergic integrity regulates microglia activity. Future studies are needed to explore the different factors that influence the degree of inflammatory activity in MDD, given the discrepancy in current evidence. The role of cholinergic system integrity in microglia response is further corroborated by our findings, but more direct in vivo measures of cholinergic system integrity derived from PET may provide confirmation and more insight into the underlying mechanisms.

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Conflicts of interest

ST participated in scientific advisory boards of Roche Pharma AG, Biogen, Grifols SA, and MSD, and received lecture fees from Roche Pharma AG and MSD.

DB, CRP, AH, JRC, RSO, and NP have no conflicts of interest to declare.

HZ has served at 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.

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, 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, all unrelated to the work presented in this paper.

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Table 1: Antidepressive treatment status in the MDD cases

curr. no.	baseline	year 1	year 3	year 3
1	✓	✓	✓	✓
2	✓	✓	✓	✓
3	✓	✓	✓	✓
4	✓	✓	✓	✓
5	✓	✓	✓	✓
6	✓	✓	✓	✓
7	✓	✓	✓	✓
8	✓	✓	✓	✓
9	✓	✓	✓	-
10	✓	✓	✓	-
11	✓	✓	✓	-
12	✓	✓	✓	-
13	✓	✓		-
14	✓	-	✓	✓
15	✓	-	-	-
16	-	✓	✓	✓
17	-	✓	✓	✓
18	-	✓	✓	✓
19	-	✓	-	
20	-	-	-	✓
21	-	-	-	-
22	-	-	-	-
23	-	-	-	-
24	-	-	-	-
25	-	-	-	-
26	-	-	-	-
27	-	-	-	-
28	-	-	-	-
29	-	-	-	-

✓ antidepressive treatment recorded for this time point

- no antidepressive treatment recorded for this time point

Table 2: Patient demographics

	MDD (*n* = 29)	**Controls (n = 20)**
Sex (female/male)[1]	9/20	12/9
Age (mean, 95% CI) [years][2]	66.7 (64.6 – 69.9)	67.8 (64.4 - 71.2)
basal forebrain/TIV (mean, 95% CI)[3]	0.42 (0.40 – 0.45)	0.41 (0.38 – 0.44)
Baseline CSF Aβ42/Aβ40 (mean, 95% CI)[4]	0.065 (0.059 - 0.071)	0.071 (0.063 - 0.080)
Baseline CSF $ptau_{181}$ (mean, 95% CI)[5]	46.26 (36.72 -55.80)	50.15 (39.32 - 60.98)
Baseline CSF tau (mean, 95% CI)[5]	314.39 (238.42 - 390.35)	343.80 (272.36 - 415.24)
Baseline CSF sTREM2 (mean, 95% CI)[6]	3507.31 (2527.65 – 4486.97)	5096.12 (3815.31 – 6376.93
3-years follow-up CSF sTREM2 (mean, 95% CI)[7]	3735.05 (2792.26 – 4677.83)	4591.78 (3333.47 – 5850.09)

MDD – major depressive disorder; CI = credibility interval.

[1]Bayes factor in favor of a group effect on sex distribution (BF_{10}) = 2.4, *i.e.*, a group effect is 2.4 times more likely than the absence of such effect.

[2]Bayes factor in favor of no group effect (BF_{10}) = 0.34; *i.e.*, absence of an effect of diagnosis on age is 1/0.34 = 2.9 times more likely than the presence of an effect.

[3]Bayes factor in favor of no group effect (BF_{10}) = 0.34; *i.e.*, absence of an effect of diagnosis on basal forebrain/TIV is 1/0.34 = 2.9 times more likely than the presence of an effect.

[4]Bayes factor in favor of no group effect (BF_{10}) = 0.57; *i.e.*, absence of an effect of diagnosis on basal forebrain/TIV is 1.8 times more likely than the presence of an effect.

[5]Bayes factor in favor of no group effect (BF_{10}) = 0.32; *i.e.*, absence of an effect of diagnosis on basal forebrain/TIV is 3.1 times less likely than the presence of an effect.

[6]Bayes factor in favor of a group effect (BF_{10}) = 1.60; *i.e.*, the presence of a group effect is 1.6 times more likely than the absence of such effect.

[7]Bayes factor in favor of no group effect (BF_{10}) = 0.53; *i.e.*, the absence of an effect of diagnosis is 1.9 times more likely than the presence of such effect.

Table 3: Basal forebrain /TIV volume and CSF sTREM2 levels in controls

Table 3a: Baseline sTREM2 levels

Model Comparison					
Models	**P(M)**	**P(M|data)**	**BF_M**	**BF_{10}**	**Error%**
Null model (incl. sex, age)	0.500	0.658	1.920	1.000	
Basal forebrain/TIV	0.500	0.342	0.521	0.521	11.854
Note. All models include sex and age					

Controlling for age and sex, absence of an effect of BF volume on baseline CSF-Trem2 levels was 1.9 times more likely than presence of an effect, i.e., anecdotal effect.

Table 3b: sTREM2 levels at three years follow-up

Model Comparison					
Models	**P(M)**	**P(M|data)**	**BF_M**	**BF_{10}**	**Error%**
Null model (incl. sex, age)	0.500	0.699	2.322	1.000	
Basal forebrain/TIV	0.500	0.301	0.431	0.431	1.008
Note. All models include sex and age					

Controlling for age and sex, absence of an effect of BF volume on baseline CSF-Trem2 levels was 2.3 times more likely than presence of an effect, i.e., anecdotal effect.

Table 4: Basal forebrain/TIV volume and CSF sTREM2 levels in MDD cases

Table 4a: Baseline sTREM2 levels

Model Comparison					
Models	**P(M)**	**P(M|data)**	**BF_M**	**BF_{10}**	**Error%**
Null model (incl. sex, age)	0.500	0.692	2.246	1.000	
Basal forebrain/TIV	0.500	0.308	0.445	0.445	1.366
Note. All models include sex and age					

Controlling for age and sex, absence of an effect of BF volume on baseline CSF-Trem2 levels was 2.2 times more likely than presence of an effect, i.e., anecdotal strength of effect.

Table 4b: sTREM2 levels at three years follow-up

Model Comparison					
Models	**P(M)**	**P(M|data)**	**BF_M**	**BF_{10}**	**Error%**
Null model (incl. sex, age)	0.500	0.112	0.127	1.000	
Basal forebrain/TIV	0.500	0.888	7.895	7.895	5.450
Note. All models include sex and age					

Controlling for age and sex, effects of basal forebrain volume on CSF-Trem2 levels at 3 years follow-up were 7.9 times more likely than absence of an effect, i.e., moderate effect.

Figure legends

Figure 1: Correlation between basal forebrain volume and CSF sTREM2 levels at three years follow-up in MDD cases

Scatter plot of CSF sTREM2 levels at 3-years follow-up (sTREM2-3rd-FU) by basal forebrain volume normalized to total intracranial volume (nBF) with least square regression line.

Figure 2: Bayes factor robustness check for the correlation between basal forebrain volume and CSF sTREM2 levels at three years follow-up in MDD

Plot of the Bayes factor corresponding to different choices of the beta prior. The data indicate that Bayes factor estimates are robust across the whole range of prior specifications.

Figure 3: Sequential analysis for the correlation between basal forebrain volume and CSF sTREM2 levels at three years follow-up in MDD

Sequential evaluation of Bayes factor with increasing number of cases in the sample. The data stabilize at a strong level of evidence after n = 16 cases have been included.

Figure 1: Correlation between basal forebrain volume and CSF sTREM2 levels at three years follow-up

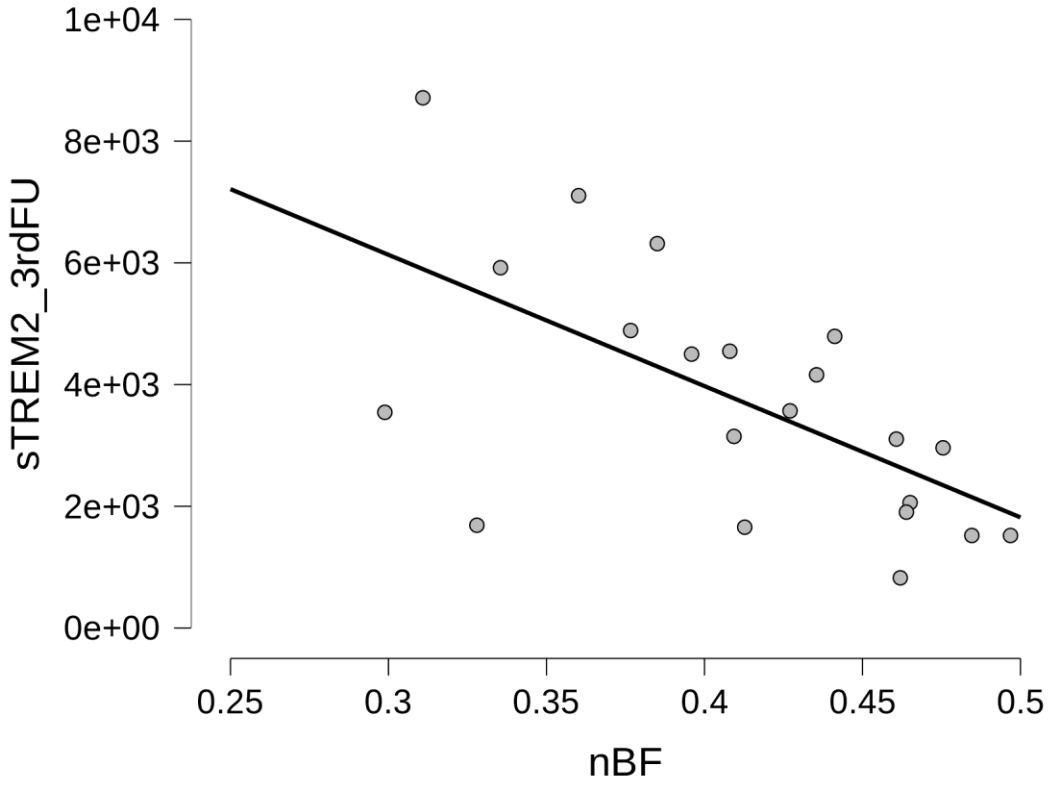

Figure 2: Bayes factor robustness check for the correlation between basal forebrain volume and CSF sTREM2 levels at three years follow-up in MDD

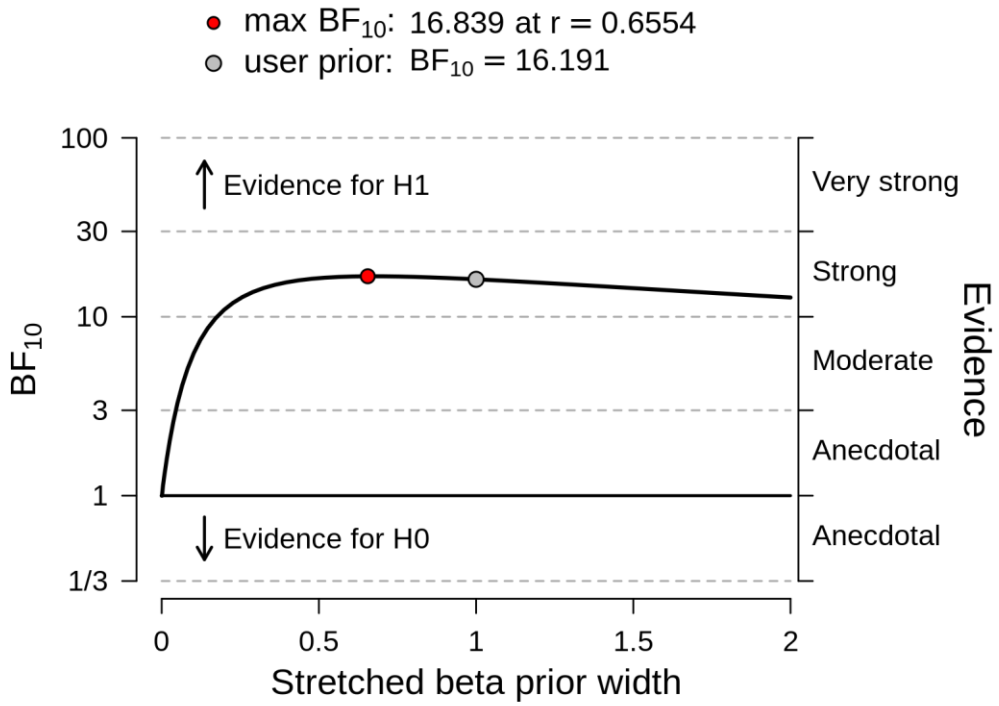

Figure 3: Sequential analysis for the correlation between basal forebrain volume and CSF sTREM2 levels at three years follow-up in MDD