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Negative results

Total glutamine synthetase levels in cerebrospinal fluid of Alzheimer's disease patients are unchanged

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ABSTRACT

Decreased cerebral protein and activity levels of glutamine synthetase (GS) have been reported for Alzheimer's disease (AD) patients. Using a recently established method, we quantified total GS levels in cerebrospinal fluid (CSF) from AD patients and control subjects. Furthermore, we investigated if total GS levels in CSF could differentiate AD from frontotemperal dementia and dementia with Lewy bodies patients. As we found no significantly altered total GS levels in any of the patient groups compared with control subjects, we conclude that levels of total GS in CSF have no diagnostic value for AD, dementia with Lewy bodies, or frontotemperal dementia.

1. Introduction

Abnormal levels of glutamine synthetase (GS), an astrocytic enzyme that regulates levels of glutamate and ammonia in the brain (Rose et al., 2013), have been reported in Alzheimer's disease (AD) brains. Both the native multimeric form and the 44 kDa monomeric form of GS (Boksha et al., 2002) have been investigated for their potential as cerebrospinal fluid (CSF) biomarkers for AD. One study demonstrated the presence of monomeric GS in 38 of 39 AD CSF samples, whereas only one of 29 control samples had a detectable level of GS (Gunnersen and Haley, 1992). In another study, using a sandwich enzyme-linked immunosorbent assay (ELISA) that does not detect monomeric GS (Tumani et al., 1995), CSF levels of multimeric GS were found increased in AD (Tumani et al., 1999).

Recently, we developed a protocol that unmasks protein epitopes by applying acidification and subsequent neutralization to CSF (AFBN protocol) (Herbert et al., 2012). The AFBN protocol was shown to dissemble multimeric GS into monomeric GS proteins and therefore allows for the quantification of total levels of GS. Applying the AFBN protocol into a homemade ELISA (Herbert et al., 2012), we now aimed to quantify levels of total GS, which—based on the previously reported increased levels of monomeric and multimeric GS—we expect to be increased in CSF samples of AD patients compared with control CSF samples. Additionally, we compared total GS levels in AD with those in frontotemporal dementia (FTD) and dementia with Lewy bodies (DLB), to investigate the biomarker potential of CSF GS in the differential diagnosis of various types of dementia. CSF levels of the established biomarkers Aβ42, total tau (t-tau), and phosphorylated tau (p-tau) were determined to study potential correlations.

2. Methods

This study included CSF samples of 26 control subjects, 34 AD patients, 21 DLB patients, and 19 FTD patients (for demographics see Table 1) obtained by lumbar puncture between 2000 and 2009. Total GS levels were measured in AFBN-pretreated CSF using a homemade ELISA as previously described (Herbert et al., 2012). Aβ42, t-tau, and p-tau levels were determined using commercially available ELISA kits (Fujirebio Europe, Ghent, Belgium). Statistical analysis on non-Gaussian distributed data was performed using Kruskal-Wallis tests with Dunn Multiple Comparison post hoc

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Table 1
Demographics and CSF protein levels of control and dementia groups given as median (25th–75th percentile)

	Controls (n = 26)	AD (n = 34)	FTD (n = 21)	DLB (n = 19)	*p*
Age (y)	65 (59–77)	72 (65–77)	65 (60–73)	73 (64–79)	NS[a]
Males (%)	62	34[‡]	67	100	<0.0001[a]
MMSE score	28 (24–29) (n = 5)	21 (19–23) (n = 29)[*]	22 (15–25) (n = 16)	24 (22–28) (n = 9)	<0.05[a]
Aβ42 (pg/mL)	789 (519–976)	449 (354–503)[*,#]	752 (573–943)	545 (400–678)[*,#]	<0.0001[b]
T-tau (pg/mL)	300 (196–390)	653 (356–736)[*,#,‡]	335 (241–401)	214 (166–290)	<0.0001[a]
P-tau (pg/mL)	57 (47–73)	106 (71–132)[*,#,‡]	58 (51–85)	46 (38–61)	<0.0001[a]
GS (ng/mL)	64 (45–108)	75 (53–95)	90 (61–127)	88 (65–122)	NS[a]

[*] $p < 0.05$ versus control, [#] $p < 0.05$ versus FTD, [‡] $p < 0.05$ versus DLB.
Key: AD, Alzheimer's disease; ANOVA, analysis of variance; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; GS, glutamine synthetase; MMSE, mini-mental state examination; NS, nonsignificant; p-tau, phosphorylated tau; SD, standard deviation; t-tau, total tau.
[a] Kruskal-Wallis test (Dunn Multiple Comparison post hoc tests).
[b] ANOVA (Bonferroni post hoc tests).

analysis. On Gaussian distributed data, 1-way ANOVA with Bonferroni post hoc analysis was performed. To investigate correlations between different markers, the Spearman method was performed. Detailed methods are provided in the Supplemental Data.

3. Results

There was no difference in age between the various groups (Table 1, $p = 0.09$), but gender distribution did differ between the groups (Table 1, $p < 0.0001$). The post hoc test revealed a difference for gender distribution between AD and DLB ($p < 0.001$). MMSE scores also differed between groups (Table 1, $p = 0.01$) with post hoc analysis revealing a lower score in AD patients versus controls, as expected ($p < 0.05$). Correlation analysis revealed no association between total GS levels in CSF and MMSE score and age or gender (data not shown).

There were no differences in total GS levels between the different patient groups and control subjects (Fig. 1 and Table 1, $p = 0.20$). Levels of the established AD biomarker Aβ42 (Table 1) were lower in AD and DLB patients compared with control subjects (both $p < 0.001$) and FTD patients ($p < 0.001$ and $p < 0.01$, respectively). There was no difference in Aβ42 levels between control subjects and FTD patients or between AD and DLB patients (Table 1). T-tau and p-tau levels were higher in AD patients compared with control subjects (both $p < 0.001$), DLB patients (both $p < 0.001$), and FTD patients ($p < 0.05$ and $p < 0.01$, respectively), while neither t-tau nor p-tau levels differed between the latter groups (Table 1).

Correlation analysis demonstrated a negative correlation between t-tau and total GS ($r = -0.45$, $p = 0.008$) and between p-tau and total GS ($r = -0.48$, $p = 0.004$) in the AD group (Supplementary Fig. 1). There was no correlation between t-tau or p-tau levels and total GS in control subjects, DLB, or FTD patients (data not shown). Total GS levels in CSF did not correlate with CSF Aβ42 levels in any group (data not shown).

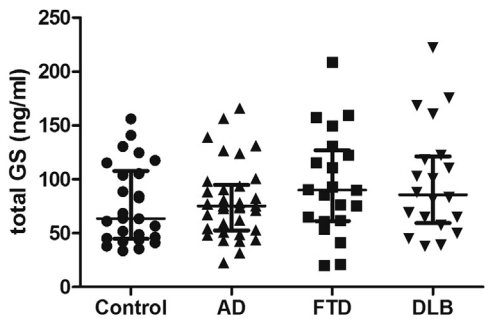

Fig. 1. Total CSF GS levels (ng/mL) in controls (n = 26) and dementia patients (AD: n = 34; FTD: n = 21; DLB: n = 19). Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; GS, glutamine synthetase.

4. Discussion

Previously, in 2 separate studies increased concentrations of both monomeric and multimeric GS were found in CSF samples of AD patients compared with nondemented control subjects (Gunnersen and Haley, 1992; Tumani et al., 1999). We now performed a quantitative measurement of total GS and found that the level of total GS in CSF did not differ between control subjects and AD patients. Furthermore, although the relative specificity of elevated (monomeric) GS levels for AD compared with other neurologic diseases (e.g., Parkinson's disease, amyotrophic lateral sclerosis, and Pick disease) was previously demonstrated (Gunnersen and Haley, 1992), we could not find a similar specificity for total GS in CSF when comparing AD with DLB or FTD. Correlation analysis revealed a negative correlation between total GS and (phosphorylated) tau levels specifically in the CSF of AD patients that may warrant further investigation.

There are a few possible explanations for the discrepancies between our study and the previous studies on GS in CSF (Gunnersen and Haley, 1992; Tumani et al., 1999). First, differences in the source of CSF may have affected the results. Although we used lumbar CSF, a previous study measured GS mostly in ventricular CSF (Gunnersen and Haley, 1992). Indeed, the number of lumbar CSF samples used in both previous studies was lower than in our study. Second, the previously reported photolabeling technique to measure monomeric GS levels (Gunnersen and Haley, 1992) is a less quantitative method than the ELISA measurements we used.

In conclusion, our study suggests that total GS levels in CSF is not a suitable biomarker for AD and does not confirm the previously suggested biomarker potential for both monomeric and multimeric GS. This finding is in line with a recent study that disproved the potential of serum GS levels as a diagnostic tool in AD (Vermeiren et al., 2011). More extensive studies may be needed to confirm the diagnostic value of either multimeric or monomeric GS for AD.

Disclosure statement

None of the authors has a conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2014.12.010.

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