Cerebrospinal fluid stanniocalcin-1: no relation to Alzheimer's disease but potentially to other dementia disorders

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ABSTRACT

Background: Stanniocalcin-1 (STC-1) is a nerve cell-enriched protein involved in intracellular calcium homeostasis regulation. Its expression increases in response to ischemic stroke but whether it is altered in neurodegeneration is unknown. The main objective of this study was to examine if STC-1 is measureable in human cerebrospinal fluid (CSF) and whether the concentration of STC-1 is increased in CSF of patients with Alzheimer's disease (AD) and other dementias, as compared to cognitively normal controls (CNC).

Method: A total of 163 individuals from 2 centers in Sweden and France were included. Cohort A consisted of 40 patients who had CSF AD biomarker patterns indicative of or excluding AD (20 patients in each group). Cohort B consisted of 43 patients, 11 of whom had AD with dementia, 12 of whom had prodromal AD, and 23 of whom had diagnoses of dementias other than AD. Cohort C included 80 patients, 32 of whom had AD, 13 stable mild cognitive impairment (MCI) and 15 with other diagnoses of dementia. Additionally, cohort C included 20 CNC.

Results: CSF STC-1 concentration was readily measureable in all CSF samples and significantly increased in neurochemical AD patients vs. neurochemically normal controls (*P*<0.0001). In cohort B, STC-1 was higher in AD vs. prodromal AD, and other dementias, but the difference was not significant when correcting for multiple group comparisons. In cohort C, there was no significant difference in CSF STC-1 concentration between AD and CNC, however, STC-1 was increased in mAD and sMCI group. STC-1 concentration was significantly lower in patients with other dementias compared to AD and CNC.

Conclusion: There was no consistent pattern of changes in CSF STC-1 between AD and control groups in three cross-sectional studies. However, consistently CSF STC-1 concentration was lower in patients with dementia diagnoses other than AD, both when comparing against AD patients and controls.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly ¹⁻ ⁴. The main histopathological hallmarks of AD are intracellular neurofibrillary tangles and extracellular amyloid plaques. The tangles are mainly composed of phosphorylated tau, while the plaques contain amyloid β (A β).^{5,6} The histopathological changes in AD are reflected in cerebrospinal fluid (CSF) as reduced levels of the 42 amino acid isoform of A β (A β 1-42), and increased level of total tau (T-tau) and phosphorylated tau (P-tau). However, as other aspects of the disease are being unraveled, CSF markers that reflect other disease mechanisms (e.g. neuronal and synaptic injury) are gaining interest as potential diagnostic and prognostic markers.^{7,8} Such markers may provide insight into the different mechanisms implicated in AD pathogenesis and assist in identifying novel targets for therapies in the future.

Stanniocalcin-1 (STC-1) is a 56-kDa homodimeric glycoprotein secreted hormone that was originally identified in bony fish, where it is believed to regulate calcium/phosphate homeostasis and protects against toxic hypercalcemia.⁹ STC-1 is expressed in various organs including neural cells, mature adipocytes, and megakaryocytes.^{10,11} High expression of STC-1 has been reported in terminally differentiated mammalian brain neurons, with the Purkinje cells, the large neurons of basal ganglia, and the pyramidal neurons in the neocortex being particularly rich in STC-1.¹² STC-1 in brain neurons plays an important role against hypercalcemic and hypoxic damage.¹³ An upregulated neuronal expression of STC-1 has been reported in penumbra areas in human and rat brain infarction.¹³

With the above in mind, we hypothesized that STC-1 would be altered in CSF of patients with AD. We specifically tested the following hypotheses: i) STC-1 is secreted from neurons, ii) that STC-1 would be possible to measure in human CSF, and iii) STC-1 levels in CSF of patients with AD would be elevated. We therefore measured STC-1 in CSF from three independently recruited cohorts,

including patients with AD and other types of dementias, as well as cognitively normal controls (CNC).

METHODS

Participants

Cohort A

Cohort A was our first explorative analysis cohort, collected at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital. The CSF samples were from patients who sought medical advice because of cognitive impairment. Patients were designated as normal (n=20) or AD (n=20) according to CSF AD biomarker concentrations using cutoffs that are around 90% specific for AD¹⁴: total tau (T-tau) >350 ng/L and A β 42 <530 ng/L. None of the biochemically normal subjects fulfilled these criteria.

Cohort B

We also included an independent cohort, enrolled at xxx site in Paris, France, consisting of a total of 43 patients (12 AD cases, 12 prodromal AD, and 19 with other dementias (OD). The OD group consisted of patients with frontotemporal dementia (FTD) (n=9), non-AD dementia (NAAD) (n=5), and 5 with clinically undetermined cognitive disorder (UCD).

Cohort C

Cohort C included a total of 80 patients: 18 AD patients, 8 patients with mixed AD and cerebrovascular disease (mAD), 13 patients with stable mild cognitive impairment (sMCI) patients, 6 prodromal AD patients, 20 CNC and 15 patients with other dementias. The other dementia group consisted of patients with vascular dementia (VaD) (n = 13), dementia of Lewy body type (DLB) (n = 4), and FTD (n =1). The study participants were recruited at the memory clinic in Falköping, Sweden, and have been described in detail elsewhere.¹⁵ The CNCs were mostly spouses of the included patients but some were recruited by advertisements in local newspapers. These individuals had no subjective symptoms of cognitive dysfunction and were cognitively normal upon formal testing.

Ethics

The Institutional Review Board for medical research at the University of Gothenburg, Sweden, and XXX, Paris, France approved the use of human subjects for these studies. Written informed consent was obtained from all participants or, if incapable, from their next of kin, prior to the inclusion in the study.

Biochemical procedure

CSF was collected in polypropylene tubes by lumbar puncture through the L3-4 or L4-5 interspace. All CSF samples were stored at -80 °C pending analysis. CSF concentrations of Aβ1-42, T-tau, and P-tau were measured with INNOTEST (Fujirebio, Ghent, Belgium). CSF concentrations of STC-1 were measured using single determinations, in a sandwich enzyme immunoassay, BioAssayTM ELISA Kit (Human), according to the instructions from the manufacturer and using two-fold dilution of the samples. All measurements were performed by board-certified laboratory technicians who were blinded to clinical data.

APOE allele

Genotyping for *APOE* (gene map locus 19q13.2) was performed using allelic discrimination technology (TaqMan; Applied Biosystems) or equivalent techniques. Genotypes were obtained for the 2 single-nucleotide polymorphisms that are used to define the ϵ_2 , ϵ_3 , and ϵ_4 alleles unambiguously (rs7412 and rs429358).

Statistical analyses

We evaluated association between demographic factors, CSF STC-1, CSF AD biomarkers and mini-mental state examination (MMSE) score using Pearson's correlation. For the group differences in sex, we used Pearson's χ^2 test. Mann-Whitney *U* test was used for pairwise comparison and for multiple group comparison Kruskal-Wallis test was used. Bonferroni correction was performed for all multiple comparisons. All tests were two-sided and statistical significance

was determined at P<0.05. All statistical analyses were performed using R (v. 3.0.3, The R Foundation for Statistical Computing).

RESULTS

Demographic characteristics of the study cohorts

The sample size and demographic characteristics of all the three cohorts are summarized in Table 1. As expected, *APOE* ε 4 prevalence was highest in patients with AD, and lowest in CNC, whereas MMSE score was lowest in patients with AD. Also, in all three cohorts, the levels of A β 1-42 were lower in AD patients, while the levels of T-tau and P-tau were significantly higher in AD (Table 1). The CSF STC-1 levels did not differ by age or sex in this cohort (Results in the Supplement).

STC-1 was readily measureable in human CSF. The detection range for the assay was 31.2-2000 pg/mL, and all the samples were almost 10-fold higher than the lowest detection range. The concentrations of the samples were in mid region of the calibration curve. The CV of the calibration curve was less than 13 %.

Increased STC-1 in neurochemically diagnosed AD cases

CSF STC-1 concentration was significantly (P < 0.0001) increased in AD patients compared with CNC in cohort A (Figure 1A).

Decreased STC-1 in other dementias versus AD and CNC

In cohort B, the levels of CSF STC-1 were increased in AD patients vs. prodromal AD and patients with other dementias, however, the increase was not statistically significant (P=0.30 and P=0.77, respectively), when corrected for multiple group comparisons (Figure 1B). In cohort C, there was no significant difference in the levels of STC-1 between AD and CNC (Figure 1C). However, the levels of STC-1 were increased in mAD and sMCI compared with CNC?. The levels of STC-1 were significantly lower in other dementias vs. CNC and sMCI (P=0.02 and P=0.04, respectively), after correcting for multiple group comparisons (Figure 1C). When all the three cohorts were combined, the levels of STC-1 were significantly (P=0.004) elevated in AD vs. other dementias after correcting for multiple group comparison. Detailed analysis of CSF STC-1 concentration among dementias other than AD, revealed significantly lower concentrations of CSF STC-1 in DLB and VaD patients (Figure 2A-B).

Correlation of CSF STC-1 with markers of AD

The CSF STC-1 levels correlated positively with T-tau (r=0.56, P<0.001) and P-tau (r=0.51, P<0.001) in cohort A, while there was a negative correlation with A β 1-42 (r= -0.30, P=0.06) (Figure 3). In cohort B, there was a positive correlation between STC-1 and T-tau, as well as P-tau (Figure 3). However, there was no significant correlation between STC-1 and T-tau in cohort C, while, there was a significant correlation with A β 1-42 and P-tau (Figure 3).

Stanniocalcin-1 levels and cognitive decline

There was no correlation between the levels of STC-1 and MMSE score in cohort B (Figure 4). However, in cohort C the levels of STC-1 correlated with MMSE score (Figure 4). There was no significant correlation between STC-1 and MMSE scores when both cohort B and C were combined (Figure 4).

The effect of APOE ε4 on CSF STC-1 concentration

In cohort C (the only cohort in which we had access to *APOE* genotype data), there was a significant association of *APOE* genotype and CSF STC-1 concentration; *APOE* ϵ 4 carriers had increased concentrations of CSF STC-1 compared with non-carriers (mean, 280 pg/mL, SD (76), vs. mean 240 pg/mL, SD (73), *P*=0.048).

DISCUSSION

The biological function of STC-1 as a key regulator of neuronal calcium homeostasis¹⁶, the potential association of dysregulated calcium homeostasis with neurodegenerative diseases,^{17,18} and the increased STC-1 expression in cerebrovascular disease^{13,19} led us to hypothesize that the STC-1 concentration in CSF could be altered in AD and/or other neurodegenerative diseases. We found that: 1) STC-1 was readily measureable in CSF using a well established ELISA, 2) CSF STC-1 concentration was significantly increased in neurochemically verified AD cohort compared to CNC, 3) there was no significant difference in the levels of STC-1 and CNC in a well-characterized AD cohort, however, the levels of STC-1 was higher in mAD and sMCI patients, 4) patients with other dementias had significantly lower levels of CSF STC-1 compared with AD dementia, prodromal AD and CNC groups, and 5) the levels of STC-1 were significantly higher in *APOE*-carrier than non-carriers.

In our exploratory cohort of neurochemically verified AD patients, the levels of STC-1 was significantly increased in AD vs. CNC. However, in cohort B, there was no significant difference in the levels of STC-1 between AD patients and prodromal AD, and in cohort C, the levels of STC-1 were lower in AD patients compared with CNC. At present, we do not have any definite explanation to why STC-1 was increased in neurochemically verified AD patients as compared to non-AD cases in cohort A, and not in the other two cohorts. However, one may speculate that the increase could be due to bias in selectivity as the diagnosis of AD was based on the results of the core AD biomarker cut-offs in this cohort with no clinical data (other than that the patients sought medical advice at a memory clinic) available. Further, the non-AD subjects in cohort A may suffer from other dementias that in the other cohorts tended to be associated with lower CSF STC-1 concentration. A replicable finding from both cohort B and C was that the levels of STC-1 were significantly lower in patients with DLB and VaD compared to CNC and AD. The mechanism of why the level of STC-1 is reduced in DLB and VaD is not fully understood. In the latter, one could speculate, based on the results from the previous experimental studies of STC-1 that cerebral or

vascular ischemia may result in higher tissue uptake of STC-1, thereby, reduced levels in CSF.^{13,20} In addition, the levels of STC-1 were significantly increased in *APOE* ε 4 carriers vs. non-carriers. Also, these findings were not consistent across the cohorts; the reason for the lack of consistency is at present unknown.

In summary, our study provides no clear support for CSF STC-1 as a biomarker for AD. This conclusion is supported by the lack of robust association of CSF STC-1 with established CSF AD biomarkers. However, one replicable result was the low CSF STC-1 concentration seen in the heterogeneous group of dementias other than AD. DLB and VaD stand out as the specific other dementia diagnosis with the lowest CSF STC-1 concentrations. Further research is warranted to examine this in closer detail and, if verified, clarify the causal relationships.

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Figure legends/Tables

Table 1. Demographic characteristics of the study population							
Characteristics	CNC	sMCI	pAD	OD	mAD	AD	
Cohort A, n	20	NA	NA	NA	NA	20	
Age at LP, mean (SD), y	69 (5)	NA	NA	NA	NA	71 (8.0)	
Sex, F:M (%F)	11 (55)	NA	NA	NA	NA	12 (60)	
Aβ1-42, pg/mL	810 (149)	NA	NA	NA	NA	467 (95)	
P-tau, pg/mL	39 (8)	NA	NA	NA	NA	112 (25)	
T-tau, pg/mL	252 (60)	NA		NA	NA	972 (302)	
Cohort B, n	NA	NA	12	19	NA	12	
Age at LP, mean (SD), y	NA	NA	76 (4)	72 (9)	NA	75 (8)	
Gender F:M (%F)	NA	NA	4 (33)	13 (56)	NA	8 (73%)	
MMSE (range 0-30)	NA	NA	25 (2)	22.1 (6.0)	NA	21 (5.)	
Aβ1-42, pg/mL	NA	NA	537 (342)	610 (217)	NA	480 (210)	
P-tau, pg/mL	NA	NA	79 (45)	50 (24)	NA	94 (53)	
T-tau, pg/mL	NA	NA	524 (296)	307 (175)	NA	621 (327)	
Cohort C, n	20	13	6	15	8	18	
Age at LP, mean (SD), y	74 (5)	71 (4)	73 (4)	74 (4)	76 (3)	73 (4)	
Gender F:M (%F)	10 (50)	8 (61)	3 (50)	5 (33)	4 (50)	10 (55)	
MMSE (range 0-30),	28 (2)	28 (1)	27 (2)	22 (6)	21 (3)	21 (4)	
<i>APOE</i> ε4, +: -, no. (% +)	4 (20)	5 (38)	1 (16)	7 (47)	3 (37)	5 (27)	
Aβ1-42, pg/mL	895 (233)	696 (216)	437 (90)	591 (313)	472 (131)	397 (114)	
P-tau, pg/mL	65 (21)	59 (23)	94 (14)	306 (89)	91 (44)	109 (32)	
T-tau, pg/mL	311 (90)	308 (109)	492 (70)	50 (16)	500 (217)	697 (245)	

All the values are presented as mean (SD). Abbreviations: AD, Alzheimer's disease; MCI, cognitive mild impairment; CNC, cognitively normal controls; sMCI, stable MCI; mAD, mixed AD; pAD, prodromal AD; OD, other dementia than AD; LP, lumbar puncture; MMSE, mini mental state examination; *APOE*, apolipoprotein E; BMI, body mass index, CSF, cerebrospinal fluid; NA, not available. *APOE* $\varepsilon 4$ + genotype was defined by the presence of at least one APOE $\varepsilon 4$ allele.



Figure 1. CSF Stanniocalcin-1 concentrations in different diagnostic groups

Stanniocalcin-1 (STC-1) was significantly elevated in neurochemically verified AD vs. controls (A). In cohort B, STC-1 was increased in AD patients compared to the prodromal AD and other dementias, however the increase was not significant when corrected for multiple group comparisons (B). In cohort C, the STC-1 was significantly lower in other dementias vs. sMCI and CNC (C). When all the three cohorts were combined, STC-1 differed significantly between other dementias and AD (D). Abbreviations: AD, Alzheimer's disease; CNC, cognitively normal individuals; mAD, mixed AD; pAD, prodromal AD; OD, other dementias; sMCI, stable mild cognitive impairment.



Figure 2. CSF Stanniocalcin-1 levels were lower in non-AD dementias.

(A) Patients with AD had increased levels of STC-1 than patients with FTD, NAAD and UCD, however, the increase was not statistically significant when corrected for multiple group comparison. (B) The levels of STC-1 were lower in patients with DLB and VaD. Abbreviations: AD, Alzheimer's disease; pAD, prodromal AD; FTD, frontotemproal dementia; NAAD, Non-AD dementia; UCD, unclear cognitive decline; mAD, mixed AD, sMCI, stable MCI, CNC, cognitively normal controls; DLB, dementia with Lewy bodies; VaD, vascular dementia.



Figure 3. Correlation with core AD biomarkers

Figure 4. Stanniocalcin levels and MMSE scores



In cohort B, Stannioclacin-1 (STC-1) levels did not correlate with mini-mental state examination (MMSE) scores, while there was a significant correlation between STC-1 and MMSE score in cohort C.