

Soluble Transcobalamin Receptor in Relation to Alzheimer's disease and Cognitive Scores

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Abstract

Introduction: The soluble transcobalamin receptor (sCD320) is present in cerebrospinal fluid and correlates with the dementia-related biomarkers phospho-tau and total-tau. Here we present data on the relation of sCD320 to Alzheimer's disease and scores of cognitive tests.

Method: Lumbar cerebrospinal fluid samples from 42 pathologically-confirmed cases of Alzheimer's disease and 25 non-demented controls were analyzed for sCD320 employing an in-house ELISA. The participants' cognitive functions were tested using the Cambridge Cognition Examination (CAMCOG) and the Mini-Mental State Examination (MMSE).

Results: There was no significant difference in the median CSF sCD320 concentration between patients and controls. The median (2.5-97.5 percentiles) sCD320 for all participants (n=67) was 15 (3-29) pmol/L. We observed a nonlinear correlation between sCD320 and cognitive scores. Spearman's correlation between sCD320 and total CAMCOG scores was 0.627 (n=16, p=0.009) for CAMCOG scores ≤ 27 , and -0.293 (n=39, p=0.071) for CAMCOG scores ≥ 68 . Spearman's correlation between sCD320 and both the low (≤ 9) and high (≥ 16) total MMSE scores was 0.274, -0.363 (n=18, 44), P=0.272, 0.016 respectively.

Conclusion: sCD320 cannot be employed as a biomarker for differentiating Alzheimer dementia patients from controls. Further studies are warranted to explore the nonlinear correlations between sCD320 and scores of cognitive function.

Introduction

Uptake of vitamin B12 (B12) by body cells demands binding of the vitamin to its circulating transporter, transcobalamin (TC), and recognition of TC-B12 (holoTC) by the receptor CD320 [1, 2]. Deficiency of this essential vitamin in nervous system is associated with cognitive impairment and neurodegenerative disease [3, 4]. The cognitive weakening is not a specific symptom restricted to specific diseases [5]. Dementia is a known syndrome of progressive, global decline in cognition functions [6]. Alzheimer's disease (AD) is the major form of dementia and is pathologically characterized by senile plaques and neurofibrillary tangles of aggregated amyloid β and dystrophic neurites containing hyperphosphorylated tau [7, 8].

Two well-known measures of global cognitive impairment are the Mini Mental State Examination (MMSE), and the Cambridge Cognitive Examination (CAMCOG). MMSE (score range from 0-30) is the most widely used brief cognitive test [9] while CAMCOG (score range from 0-107) is intermediate in length and is designed to cover seven areas of cognitive function: orientation, language, memory, attention, praxis, abstract thinking and perception [10].

We previously demonstrated the presence of a soluble form of CD320 (sCD320) in human CSF employing an in-house ELISA and showed its correlation to dementia related biomarkers phospho-tau and total-tau [11-13]. Also our previous results suggested that CSF sCD320 is derived from local production rather than transferred from the circulation [11]. These results drive our interest to investigate in the current study the ability of sCD320 to differentiate AD from controls and how it relates to cognitive scores.

Participants and Methods

The Oxford Project To Investigate Memory and Ageing (OPTIMA) is a longitudinal study which started in 1988 and has since recruited more than 1000 elderly participants from the Oxford region, including both cases with cognitive problems and controls [14]. OPTIMA clinical protocols [15] were approved by the local ethics committee and informed consent was obtained for all individuals included in the study.

At enrollment, all subjects underwent clinical examination, and cognitive assessment. The cognitive assessment included CAMCOG and MMSE, which are the cognitive components of the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) [10]. The clinical diagnosis of AD was made according to NINCDS criteria [16] and confirmed histopathologically according to CERAD protocol [17].

Both patients and controls underwent lumbar puncture for CSF specimen collection employing standard clinical techniques [18]. CSF specimens were centrifuged for 10 min at 1,000 g at 4°C to remove cellular components and stored at –80°C until analysis for the present study. The CSF samples of 42 pathologically-confirmed cases of Alzheimer's disease (22 males and 20 females) and 25 non-demented control individuals (12 males and 13 females) were selected randomly out of the sample pool of the OPTIMA longitudinal cohort.

The samples were analyzed blinded to the clinical outcomes. We measured the CSF sCD320 employing an in house ELISA as previously described [11-13]. The inter-assay imprecision for control samples with a mean concentration of 17 pmol/L (25 runs over a period of 4 months) was 8.0% and the intra-assay imprecision was 4.3% [13].

Statistical analysis

sCD320 and cognitive scores did not follow normal distribution (using Kolmogorov-Smirnov normality tests). Thus, non-parametric statistical tests were used and measurements were reported as medians with 2.5-97.5 percentiles. Mann-Whitney U test was applied for testing the difference between median levels. Spearman's rank test was used to investigate the correlations between study variables. Spearman's correlation, like most other types of correlation tests, is unsuitable for nonlinear correlations. We observed biphasic correlations, and our choice was to apply the Spearman's correlation before and after the inflection points. Outliers are not excluded as we used statistical methods that minimize the influence of outliers. Statistical analyses were performed using SPSS statistical computer software (version 20, IBM Inc.).

Results

We explored the CSF level of sCD320 in patients with Alzheimer's disease as compared to non-demented controls. Population characteristics are indicated in table 1.

There was no significant difference in the median CSF sCD320 concentration between patients (15 pmol/L) and controls (15 pmol/L), $p=0.517$, figure 1. The median (2.5-97.5 percentiles) sCD320 for all participants ($n=67$) is 15 (3-29) pmol/L, a similar value to what we observed previously (14 pmol) in anonymized CSF samples ($n=223$) from the routine clinical laboratory [11].

We observed a nonlinear correlation between sCD320 and cognitive scores. Spearman's correlation before the first inflection point for MMSE (≤ 9) is 0.274 ($n=18$, $p=0.272$) and -0.363 ($n=44$, $p=0.016$) after the second inflection point (≥ 16) (figure 2). Spearman's correlation before the first inflection point of CAMCOG (≤ 27) is 0.627 ($n=16$, $p=0.009$) and -0.293 ($n=39$, $p=0.071$) after the second inflection point (≥ 68) (figure 3).

The source of the correlation pattern between sCD320 and CAMCOG is attributed to certain CAMCOG sub-scores mainly perception, abstract thinking, praxis, attention, and expression as can be concluded from figure 4.

No correlation was found between sCD320 and samples storing time. That indicate a suitable stability over time and is in agreement with our previous testing that sCD320 is stable after freezing and repeated thaw-freeze cycles [13].

Discussion

In this study we explore the ability of the soluble transcobalamin receptor (sCD320) to differentiate AD from normal subjects and investigate its relation to cognitive scores.

We previously showed sCD320 to correlate strongly with tau proteins [11], one of the biomarkers employed for diagnosing AD [23]. That made us question whether sCD320 is able to differentiate AD from controls. Our results show this not to be the case, but at the same time we observe an interesting relationship between sCD320 and cognitive scores.

The absent of difference in sCD320 level between AD and non-demented controls may be attributed to the biphasic correlation between sCD320 and total cognitive score. sCD320 tend to increase with mild cognitive impairment but decrease with sever cognitive impairment which give an overall picture of no difference in the level of sCD320 medians between AD and normal individuals. Due to limited samples number, we are unable to do sub-analysis to compare the sCD320 levels between non-demented individuals and AD patients grouped according to different cognitive scores. The concurrence of cognitive state deterioration (from normal to mild) with increasing level of sCD320 is in agreement with previously reported relation between tau proteins and sCD320 [11]. Therefore, sCD320 may be more useful in differentiating early dementia or early cognitive impairment from normal but of no value if the cognitive impairment is severe.

It's a challenge to explain how sCD320 is linked to the cognitive scores. sCD320 is part of the vitamin B12 system [13], where deficiency of the vitamin lead to cognitive impairments[3], but as we still lack the knowledge about the role of sCD320 within that system , no straightforward link between sCD320 and cognitive score can be stated. Also sCD320 is correlated to tau proteins and to holoTC [11, 12], both are shown previously to be linked to cognitive scores [19-22]. The nonlinear correlation between sCD320 and

cognitive scores is similar to the previously reported nonlinear correlation of cognitive scores to both phospho-tau and total-tau especially in the higher cognitive scores segment [20].

In summary we reveal a nonlinear relationship between sCD320 and cognitive scores, with tendency of positive correlation with the lower cognitive scores and negative correlation with the higher cognitive scores. Our results warrants further studies in order to explore the nonlinear correlations between sCD320 and scores of cognitive functions test.

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References

1. Quadros, E.V., Y. Nakayama, and J.M. Sequeira, *The protein and the gene encoding the receptor for the cellular uptake of transcobalamin-bound cobalamin*. Blood, 2009. **113**(1): p. 186-92.
2. Nielsen, M.J., et al., *Vitamin B12 transport from food to the body's cells--a sophisticated, multistep pathway*. Nat Rev Gastroenterol Hepatol, 2012. **9**(6): p. 345-54.
3. Tangney, C.C., et al., *Vitamin B12, cognition, and brain MRI measures: a cross-sectional examination*. Neurology, 2011. **77**(13): p. 1276-82.
4. Reynolds, E., *Vitamin B12, folic acid, and the nervous system*. Lancet Neurol, 2006. **5**(11): p. 949-60.
5. De Jager, C.A., et al., *Sensitivity and specificity of neuropsychological tests for mild cognitive impairment, vascular cognitive impairment and Alzheimer's disease*. Psychol Med, 2003. **33**(6): p. 1039-50.
6. Ariogul, S., et al., *Vitamin B12, folate, homocysteine and dementia: are they really related?* Arch Gerontol Geriatr, 2005. **40**(2): p. 139-46.
7. Karantzoulis, S. and J.E. Galvin, *Distinguishing Alzheimer's disease from other major forms of dementia*. Expert Rev Neurother, 2011. **11**(11): p. 1579-91.
8. Rijal Upadhaya, A., et al., *Biochemical stages of amyloid-beta peptide aggregation and accumulation in the human brain and their association with symptomatic and pathologically preclinical Alzheimer's disease*. Brain, 2014. **137**(Pt 3): p. 887-903.
9. Folstein, M.F., S.E. Folstein, and P.R. McHugh, *"Mini-mental state". A practical method for grading the cognitive state of patients for the clinician*. J Psychiatr Res, 1975. **12**(3): p. 189-98.
10. Roth, M., et al., *CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia*. Br J Psychiatry, 1986. **149**: p. 698-709.
11. Abuyaman, O. and E. Nexø, *The soluble transcobalamin receptor (sCD320) is present in cerebrospinal fluid and correlates to dementia-related biomarkers tau proteins and amyloid-beta*. Scand J Clin Lab Invest, 2015. **75**(6): p. 514-8.
12. Abuyaman, O., et al., *The soluble receptor for vitamin B12 uptake (sCD320) increases during pregnancy and occurs in higher concentration in urine than in serum*. PLoS One, 2013. **8**(8): p. e73110.
13. Arendt, J.F., E.V. Quadros, and E. Nexø, *Soluble transcobalamin receptor, sCD320, is present in human serum and relates to serum cobalamin - establishment and validation of an ELISA*. Clin Chem Lab Med, 2012. **50**(3): p. 515-9.
14. Hogervorst, E., et al., *Diagnosing dementia: interrater reliability assessment and accuracy of the NINCDS/ADRDA criteria versus CERAD histopathological criteria for Alzheimer's disease*. Dement Geriatr Cogn Disord, 2000. **11**(2): p. 107-13.
15. Clarke, R., et al., *Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease*. Arch Neurol, 1998. **55**(11): p. 1449-55.
16. Tamaoka, A., *[Alzheimer's disease: definition and National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)]*. Nihon Rinsho, 2011. **69 Suppl 10**(Pt 2): p. 240-5.
17. Murayama, S. and Y. Saito, *Neuropathological diagnostic criteria for Alzheimer's disease*. Neuropathology, 2004. **24**(3): p. 254-60.
18. Hindley, N.J., et al., *High acceptability and low morbidity of diagnostic lumbar puncture in elderly subjects of mixed cognitive status*. Acta Neurol Scand, 1995. **91**(5): p. 405-11.
19. Tsolaki, M., et al., *Correlation of rCBF (SPECT), CSF tau, and cognitive function in patients with dementia of the Alzheimer's type, other types of dementia, and control subjects*. Am J Alzheimers Dis Other Demen, 2001. **16**(1): p. 21-31.

20. Williams, J.H., et al., *Non-linear relationships of cerebrospinal fluid biomarker levels with cognitive function: an observational study*. *Alzheimers Res Ther*, 2011. **3**(1): p. 5.
21. Stomrud, E., et al., *Cerebrospinal fluid biomarkers predict decline in subjective cognitive function over 3 years in healthy elderly*. *Dement Geriatr Cogn Disord*, 2007. **24**(2): p. 118-24.
22. Garrod, M.G., et al., *Fraction of total plasma vitamin B12 bound to transcobalamin correlates with cognitive function in elderly Latinos with depressive symptoms*. *Clin Chem*, 2008. **54**(7): p. 1210-7.
23. Tapiola, T, et al., *Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain*. *Arch Neurol*, 2009. **66**(3):p.382-9.

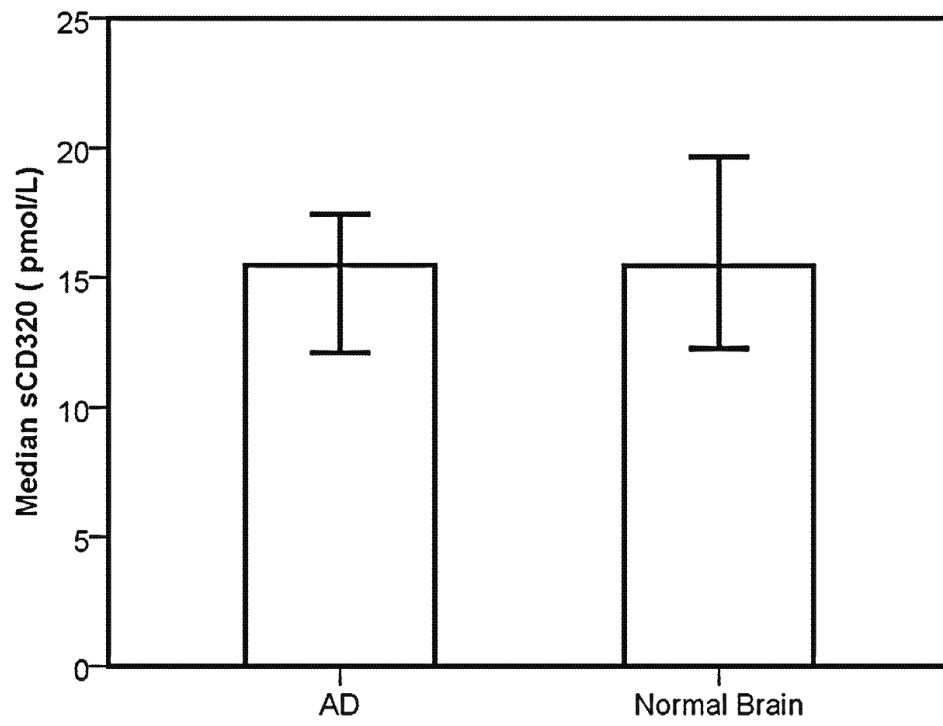


Figure 1. sCD320 concentration comparison of CSF samples from 42 AD patients and 25 non-demented participants. Median with 95% CI is indicated.

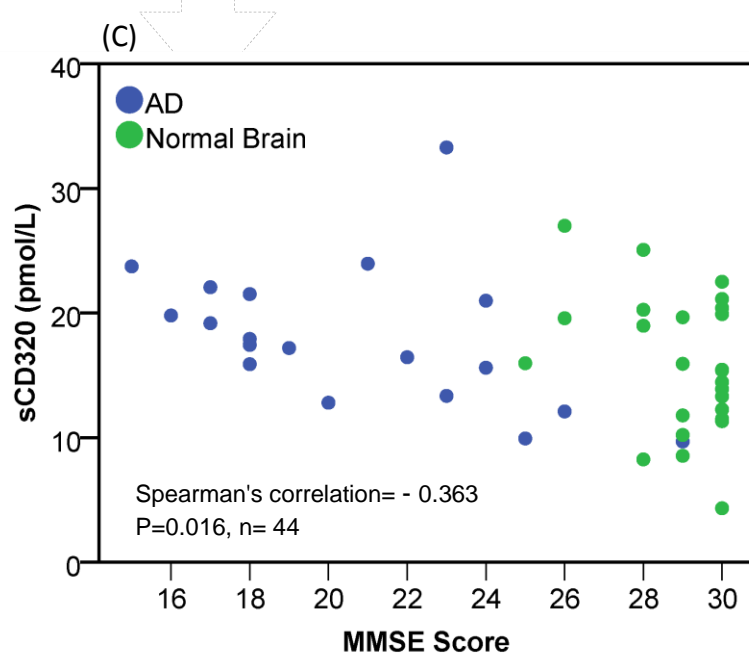
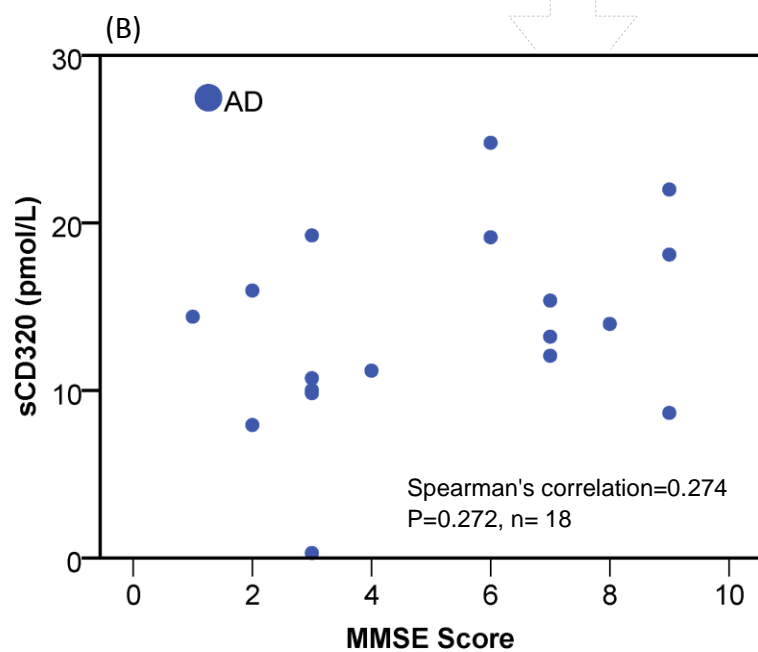
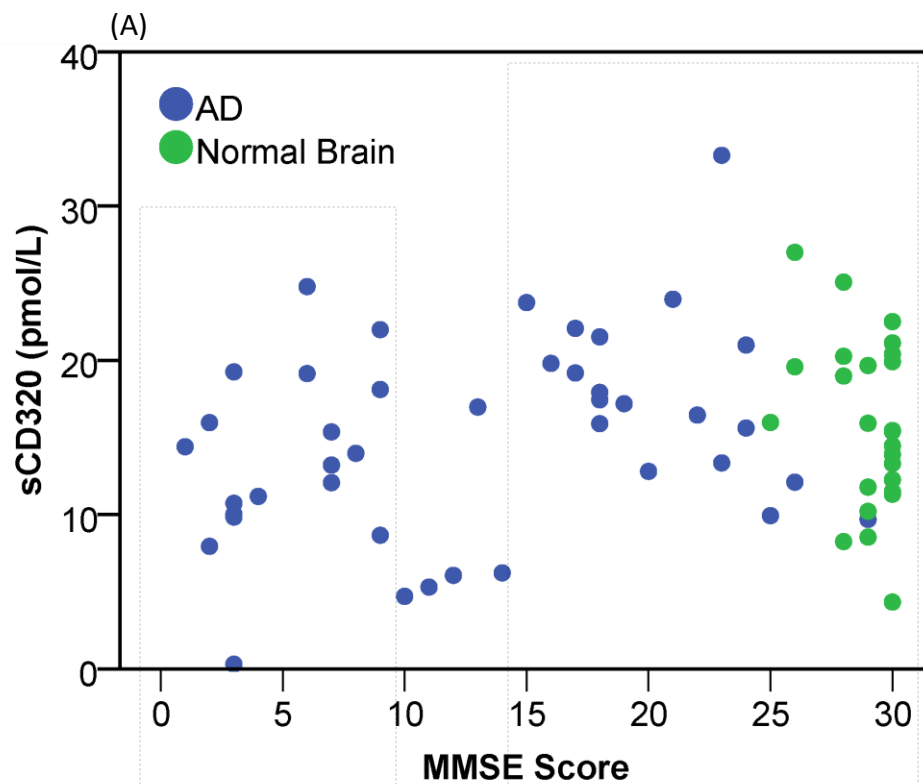


Figure 2. sCD320 correlations with MMSE total scores. The figures are based on results from 42 AD patients and 25 normal participants (A). (B) and (C) represent a zoom-in to the lower and higher end of the MMSE total score respectively. P-values obtained using spearman's correlation.

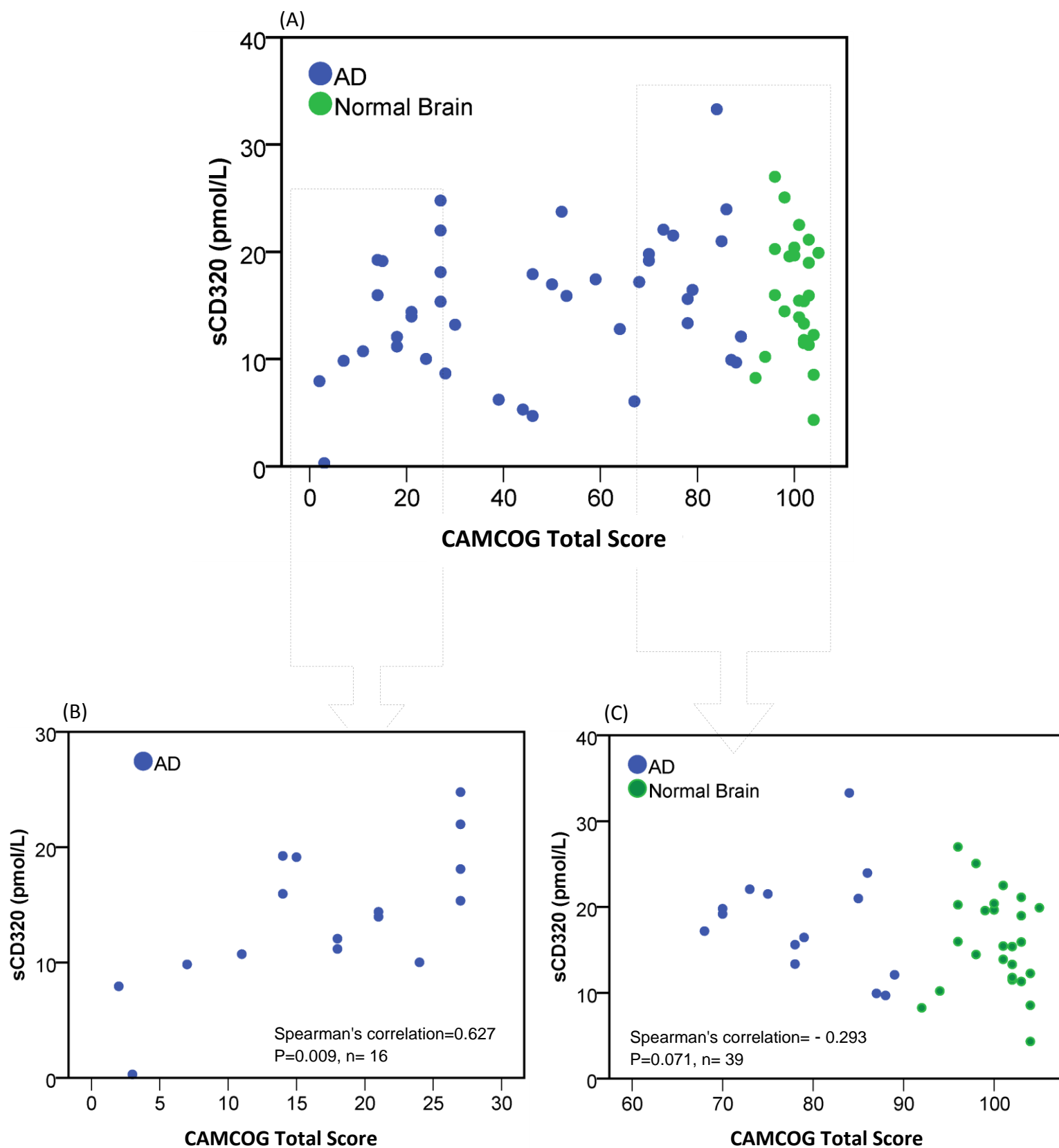


Figure 3. sCD320 correlations with CAMCOG total scores. The figures are based on results from 42 AD patients and 25 normal participants (A). (B) and (C) represent a zoom to the lower and higher end of the CAMCOG total score respectively. P-values obtained using spearman's correlation.

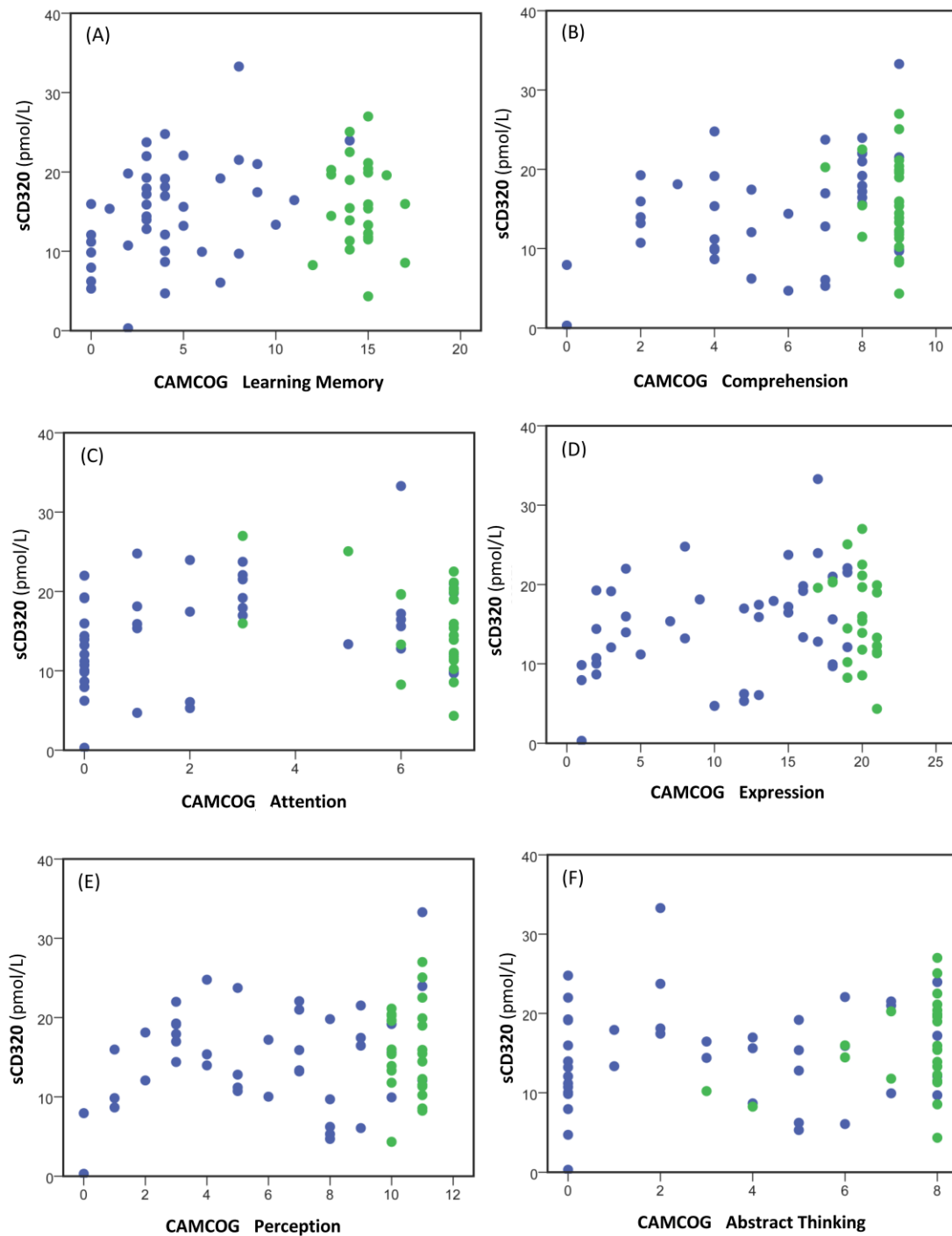


Figure 4. sCD320 correlations with CAMCOG subtests in AD patients and controls. The figures are based on results from 42 AD patients (blue) and 25 normal participants (green). Correlations of CSF sCD320 to CAMCOG subtest of learning memory (A), Comprehension (B), attention (C), Expression (D), perception (E) and abstract thinking (F).

Table 1. Basic characteristics of study participants.

	Controls	Cases	p-value
Number	25	42	-
Gender	12 male, 13 female	22 male, 20 female	-
Age, years median (range)	?? (??-??)	?? (??-??)	??
Sampling date	1990-2006	1990-2008	-

P value is according to Mann-Whitney test. Controls are non-demented individuals. Cases are patients with pathologically-confirmed Alzheimer's disease.