

Myo-inositol changes precede amyloid pathology and relate to *APOE* genotype in Alzheimer disease

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Olga Voevodskaya
 Pia C. Sundgren, MD,
 PhD
 Olof Strandberg, PhD
 Henrik Zetterberg, MD,
 PhD
 Lennart Minthon, MD,
 PhD
 Kaj Blennow, MD, PhD
 Lars-Olof Wahlund, MD,
 PhD
 Eric Westman, PhD
 Oskar Hansson, MD,
 PhD
 For the Swedish
 BioFINDER study
 group

Correspondence to
 Ms. Voevodskaya:
 olga.voevodskaya@ki.se
 or Dr. Hansson:
 oskar.hansson@med.lu.se

ABSTRACT

Objective: We aimed to test whether in vivo levels of magnetic resonance spectroscopy (MRS) metabolites myo-inositol (ml), N-acetylaspartate (NAA), and choline are abnormal already during pre-clinical Alzheimer disease (AD), relating these changes to amyloid or tau pathology, and functional connectivity.

Methods: In this cross-sectional multicenter study (a subset of the prospective Swedish BioFINDER study), we included 4 groups, representing the different stages of predementia AD: (1) cognitively healthy elderly with normal CSF β -amyloid 42 (A β 42), (2) cognitively healthy elderly with abnormal CSF A β 42, (3) patients with subjective cognitive decline and abnormal CSF A β 42, (4) patients with mild cognitive decline and abnormal CSF A β 42 ($N_{\text{total}} = 352$). Spectroscopic markers measured in the posterior cingulate/precuneus were considered alongside known disease biomarkers: CSF A β 42, phosphorylated tau, total tau, [^{18}F]-flutemetamol PET, f-MRI, and the genetic risk factor *APOE*.

Results: Amyloid-positive cognitively healthy participants showed a significant increase in ml/creatinine and ml/NAA levels compared to amyloid-negative healthy elderly ($p < 0.05$). In amyloid-positive healthy elderly, ml/creatinine and ml/NAA correlated with cortical retention of [^{18}F] flutemetamol tracer ($\hat{\beta} = 0.44, p = 0.02$ and $\hat{\beta} = 0.51, p = 0.01$, respectively). Healthy elderly *APOE* $\epsilon 4$ carriers with normal CSF A β 42 levels had significantly higher ml/creatinine levels ($p < 0.001$) than $\epsilon 4$ noncarriers. Finally, elevated ml/creatinine was associated with decreased functional connectivity within the default mode network ($r_{\text{pearson}} = -0.16, p = 0.02$), independently of amyloid pathology.

Conclusions: ml levels are elevated already at asymptomatic stages of AD. Moreover, ml/creatinine concentrations were increased in healthy *APOE* $\epsilon 4$ carriers with normal CSF A β 42 levels, suggesting that ml levels may reveal regional brain consequences of *APOE* $\epsilon 4$ before detectable amyloid pathology. **Neurology® 2016;86:1754-1761**

GLOSSARY

AD = Alzheimer disease; **Cho** = choline; **Cr** = creatine; **DMN** = default mode network; **fMRI** = functional MRI; **GLM** = general linear model; **MCI** = mild cognitive impairment; **ml** = myo-inositol; **MMSE** = Mini-Mental State Examination; **MRS** = magnetic resonance spectroscopy; **NAA** = N-acetylaspartate; **PCC** = posterior cingulate cortex; **SCD** = subjective cognitive decline; **t-tau** = total tau; **VOI** = volume of interest.

Potential biomarkers for predicting onset and progression of Alzheimer disease (AD) can be detected by proton magnetic resonance spectroscopy (MRS)—a noninvasive imaging technique that allows in vivo assessment of brain biochemistry. Decreased levels of neural marker N-acetylaspartate (NAA) and increased concentrations of myo-inositol (mI) belong to the more consistent MRS findings in patients with AD dementia.¹⁻⁴

From Clinical Geriatrics (O.V., L.-O.W., E.W.), Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Stockholm; Department of Diagnostic Radiology (P.C.S., O.S.), Lund University; Clinical Neurochemistry Laboratory (H.Z., K.B.), Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; UCL Institute of Neurology (H.Z.), Queen Square, London, UK; Memory Clinic (L.M., O.H.), Skåne University Hospital; and Clinical Memory Research Unit (L.M., O.H.), Department of Clinical Sciences, Malmö, Lund University, Sweden.

Coinvestigators are listed on the *Neurology*® Web site at Neurology.org.

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To understand the underlying causes of the observed abnormalities in the MRS neurochemical profile in AD, it is important to study their association with known pathologic processes in individuals at different disease stages. We hypothesized that at the early predementia stages, incipient AD pathology is responsible for MRS metabolite abnormalities.

We aimed to evaluate the relationship between MRS-detected brain metabolites and known AD biomarkers in the following 4 predementia groups: healthy controls with no evidence of amyloid pathology, healthy controls with abnormal levels of CSF A β 42, patients with subjective cognitive decline (SCD) with abnormal A β 42 levels, and patients with mild cognitive impairment (MCI) with abnormal A β 42 levels.

In these groups, we studied the relationship among metabolites NAA, mI, and choline (Cho) in the posterior cingulate cortex (PCC)/precuneus and A β deposition measured by [18 F]-flutemetamol PET or CSF A β 42 as well as the CSF neurodegenerative markers total tau (τ -tau) and phosphorylated tau. In addition, we evaluated MRS metabolites in relation to (1) carriership of the *APOE* ϵ 4 allele—the main known genetic risk factor for AD⁵ and (2) functional connectivity in the PCC/precuneus measured using functional MRI (fMRI), due to the default mode network (DMN) involvement in predementia AD.⁶

METHODS **Standard protocol approvals, registrations, and patient consents.** All participants gave written consent to participate in the study. Ethical approval was given by the ethical committee of Lund University, Sweden. [18 F]-flutemetamol PET imaging approval was obtained from the Swedish Medicines and Products Agency and the local Radiation Safety Committee at Skåne University Hospital, Sweden.

Participants. The study population stemmed from the prospective and longitudinal Swedish BioFinder study (more information available at www.biofinder.se and the coinvestigator list on the *Neurology*[®] Web site at Neurology.org). Among others, the BioFinder consecutively enrolls (1) cognitively healthy elderly participants and (2) patients without dementia with mild cognitive symptoms. Individuals from these 2 cohorts were selected for the present study. Data were collected between 2009 and 2014 in accordance with a standardized protocol.

The first cohort consisted of cognitively normal elderly participants who were eligible for inclusion if they (1) were aged \geq 60 years old, (2) scored 28–30 points on Mini-Mental State Examination (MMSE) at the screening visit, (3) did not have any subjective cognitive impairment, and (4) were fluent in Swedish.

Exclusion criteria included presence of significant neurologic disease (e.g., stroke, Parkinson disease, multiple sclerosis), severe psychiatric disease (e.g., severe depression or psychotic syndromes), dementia, or MCI.

The second cohort contained patients who were enrolled consecutively at 3 memory outpatient clinics in Sweden. They were referred for assessment of cognitive complaints and assessed by physicians with special interest in dementia disorders. The inclusion criteria were as follows: (1) referred to the memory clinics because of cognitive impairment; (2) not fulfilling the criteria for dementia; (3) MMSE score of 24–30 points; (4) age 60–80 years; and (5) fluent in Swedish. The exclusion criteria were (1) cognitive impairment without doubt explained by another condition (other than prodromal dementia); (2) severe somatic disease; and (3) refusing lumbar puncture or neuropsychological investigation. Further, patients were classified into MCI and SCD based on a neuropsychological battery assessing the cognitive domains of verbal ability, visuospatial construction, episodic memory, and executive functions and the clinical assessment of a senior neuropsychologist.

For the current study, only participants with CSF analysis and a high-quality MRS spectrum were eligible (see figure e-1 for flowchart). This resulted in the following 4 groups: from cohort 1, (1) healthy controls with normal (negative) CSF A β 42 ($n = 156$) and (2) healthy controls with abnormal (positive) CSF A β 42 ($n = 59$); from cohort 2, (3) patients with SCD with abnormal (positive) CSF A β 42 ($n = 49$); and (4) patients with MCI with abnormal (positive) CSF A β 42 ($n = 88$).

CSF A β 42 levels at and below 530 ng/L were considered abnormal.⁷

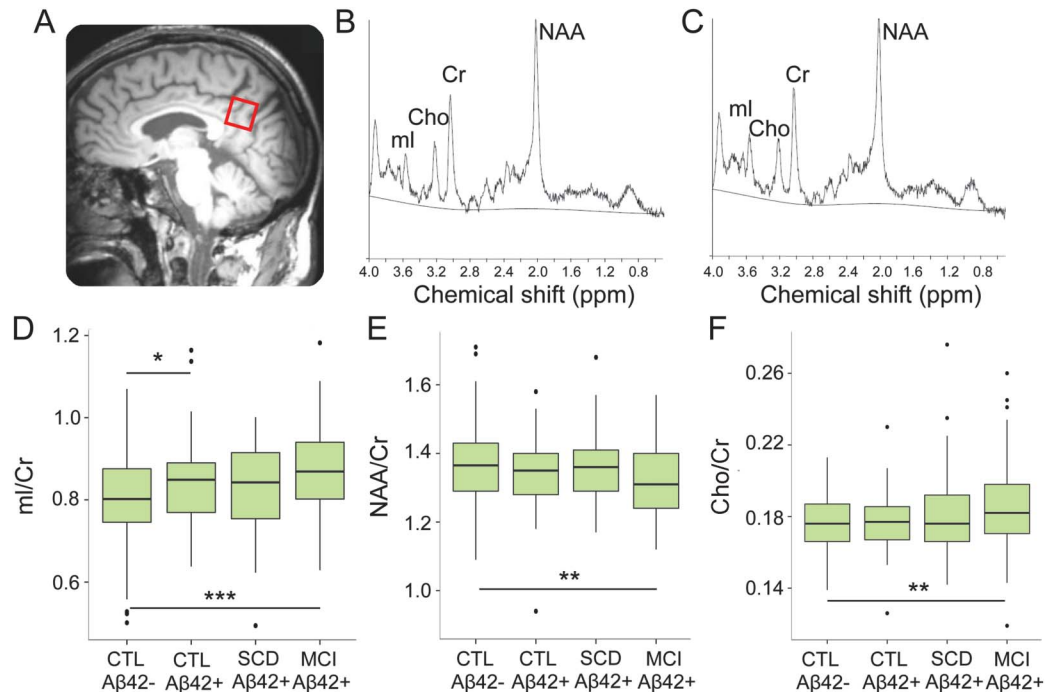
MRS acquisition. Single-voxel MRS was performed at 3T on a Siemens TrioTim scanner; the point-resolved spectroscopy sequence was applied at echo time of 30 ms and repetition time of 2,000 ms. The $2 \times 2 \times 2$ cm³ voxel was placed midsagittally in the PCC/precuneus area (figure 1). This region demonstrates histopathologic changes,⁸ cortical thinning,⁹ and decreased glucose metabolism¹⁰ early in the disease course. The PCC/precuneus area has previously been selected for MRS voxel placement in large-scale spectroscopy studies^{11,12} and was recommended for MRS studies in AD by the MRS consensus group.¹³

For details on structural MRI and resting-state fMRI, see appendix e-1.

MRS analysis. Metabolite quantification was carried out using the LCModel software¹⁴ relative total creatine (Cr) concentration (a resonance peak, composed of the metabolites creatine and phosphocreatine). This means of internal referencing is often used in clinical spectroscopy due to the relative stability of the Cr peak.¹⁵ All processed spectra were visually inspected for quality and artefacts. Only spectra with full width at half maximum \leq 11 Hz were considered. For complete quality control procedures, see appendix e-1. Examples of spectra can be found in figure 1.

Resting-state fMRI analysis. Initial preprocessing of resting-state fMRI data was performed with an FSL-based pipeline.¹⁶ A 15-mm-diameter sphere centered in the MRS region of interest was used as a seed. Correlation maps were obtained between the seeds' average and all gray matter voxels' processed blood oxygenation level–dependent time series. A normal connectivity mask was defined by averaging the maps of all A β 42-negative controls and applying a correlation threshold of approximately 0.2 (corresponding to $p < 0.01$). Participant-specific functional connectivity summary statistic was extracted as the sum of all seed

Figure 1 Voxel placement, example spectra, and metabolite levels across diagnostic groups



(A) Single voxel prescribed midsagittally on a T1-weighted image. Examples of spectra obtained from the $2 \times 2 \times 2 \text{ cm}^3$ voxel collected at echo time 30 ms in (B) a cognitively healthy control and (C) a patient with mild cognitive impairment (MCI). Metabolite levels across different stages of predementia Alzheimer disease (D-F). (D) Myo-inositol (ml)/creatine (Cr) ratio levels across diagnostic subgroups. (E) N-acetylaspartate (NAA)/Cr ratio levels across diagnostic subgroups. (F) Choline (Cho)/Cr ratio levels across diagnostic subgroups. Tukey honestly significant difference tests were used for post hoc comparisons. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CTL A β 42 $-$ = controls with CSF A β 42 >530 ng/L; CTL A β 42 $+$ = controls with CSF A β 42 ≤ 530 ng/L; SCD A β 42 $+$ = patients with subjective cognitive decline with CSF A β 42 ≤ 530 ng/L; MCI A β 42 $+$ = patients with MCI with CSF A β 42 ≤ 530 ng/L.

correlation values within the normal connectivity mask. For detailed description of fMRI procedures, see appendix e-1.

PET acquisition and analysis. The cerebral A β burden of the patients was visualized using [^{18}F]-flutemetamol PET.¹⁷ Images were analyzed using the software NeuroMarQ provided by GE Healthcare. A volume of interest (VOI) template for different cortical and subcortical regions was applied. The following 9 bilateral regions were used in the study: prefrontal, parietal, lateral temporal, medial temporal, sensorimotor, occipital, anterior cingulate, PCC/precuneus, and a global neocortical composite region.¹⁸ The standardized uptake value ratio was defined as the regional tracer uptake in a VOI, normalized for the mean uptake in the cerebellar cortex. For details on PET procedures, see appendix e-1.

CSF collection and analysis. The procedure and analysis of the CSF followed the Alzheimer's Association Flow Chart for CSF biomarkers.¹⁹ Lumbar CSF samples were collected at 3 centers, stored in polypropylene tubes at -80°C , and analyzed at the same time using 2 different ELISAs. CSF A β 42 and tau phosphorylated at Thr181 were analyzed by INNOTEST ELISAs (Fujirebio Europe, Ghent, Belgium).²⁰ CSF t-tau was analyzed by EUROIMMUN ELISA (EUROIMMUN AG, Lübeck, Germany).

Statistical procedures. Statistical analyses were performed within the general linear model (GLM) framework. Associations between MRS metabolites and other variables were assessed using

multiple stepwise linear regression with backward elimination. Thus, age and sex were included in the model and their partial effects accounted for only when these variables were demonstrated to be significant predictors.

Between-group differences were tested using independent-samples t test or using analysis of variance followed by Tukey honestly significant difference test for post hoc comparisons. χ^2 test was used to analyze categorical variables. Cohen d was used to measure effect size. For estimating the association between ml/Cr and functional connectivity, Pearson correlation coefficients were calculated.

Due to the putative neuroprotective effect of the *APOE* $\epsilon 2$ allele, participants carrying at least one $\epsilon 2$ allele were excluded from statistical analyses of genotype data.

Statistical analyses were performed using SPSS version 22 and R (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

RESULTS Sample characteristics. Demographic details of the diagnostic groups are provided in table 1 (additional cognitive tests in table e-1).

MRS, MRI, and CSF data were available for all participants, whereas PET and fMRI data were available for a subset ($N_{\text{PET}} = 166$, $N_{\text{fMRI}} = 206$). An overview of modalities per diagnostic group is available in table e-2.

Table 1 Study sample demographics

	CTL CSF A β 42 ⁻ (n = 156)	CTL CSF A β 42 ⁺ (n = 59)	SCD CSF A β 42 ⁺ (n = 49)	MCI CSF A β 42 ⁺ (n = 88)	p Value
Female/male	95/61	36/23	27/22	43/45	0.283
Age, y	72.6 (4.7)	72.7 (4.7)	70.7 (5.7)	71.7 (5.2)	0.091
APOE genotype					
ϵ 4 carriers/ ϵ 3 homozygotes	20/93 ^{a,b,c}	30/19	32/13	60/21	<0.001
ϵ 4 carriers, %	18	61	71	74	
MMSE	29.1 (0.9) ^{b,c}	29.2 (0.9) ^{b,c}	28.0 (1.6) ^{a,c,d}	26.9 (1.7) ^{a,b,d}	<0.001
Years of education	12.1 (3.7)	11.7 (3.8)	12.1 (3.8)	11.6 (3.3)	0.725
CSF A β 42, ng/L	753 (127) ^{a,b,c}	416 (75) ^{c,d}	384 (79) ^d	359 (84) ^{a,d}	<0.001
CSF tau, ng/L	125 (63) ^{a,b,c}	164 (103) ^{b,c,d}	231 (95) ^{a,d}	209 (100) ^{a,d}	<0.001
CSF p-tau, ng/L	51 (15) ^{b,c}	58 (25) ^{b,c}	77 (31) ^{a,d}	74 (36) ^{a,d}	<0.001
PET (PCC/precuneus SUVR)	1.26 (0.15) ^{a,b,c}	1.74 (0.41) ^{c,d}	1.96 (0.40) ^{c,d}	2.19 (0.47) ^{a,d}	<0.001

Abbreviations: MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; p-tau = phosphorylated tau; PCC = posterior cingulate cortex; SCD = subjective cognitive decline; SUVR = standardized uptake value ratio.

Values are reported as mean (SD).

APOE is missing for 24 participants, education data are missing for 34 participants, PET data are missing for 168 participants.

^aSignificantly different from CTL CSF A β 42⁺ ($p < 0.05$).

^bSignificantly different from SCD CSF A β 42⁺ ($p < 0.05$).

^cSignificantly different from MCI CSF A β 42⁺ ($p < 0.05$).

^dSignificantly different from CTL CSF A β 42⁻ ($p < 0.05$).

CTL CSF A β 42⁻ = healthy controls with CSF A β 42 >530 ng/L; CTL CSF A β 42⁺ = healthy controls with CSF A β 42 \leq 530 ng/L; SCD CSF A β 42⁺ = SCD participants with CSF A β 42 \leq 530 ng/L; MCI CSF A β 42⁺ = MCI participants with CSF A β 42 \leq 530 ng/L.

MRS in different stages of predementia AD. MRS outcome variables mI/Cr, NAA/Cr, and Cho/Cr measured in the PCC/precuneus were assessed across the 4 diagnostic groups. For a summary of metabolite concentrations and an account of group differences, see figure 1 and table e-3.

All spectroscopic measures were significantly different between the CSF A β 42-negative controls and the CSF A β 42-positive MCI group: mI/Cr was elevated ($p < 0.001$, d [Cohen] = 0.64), Cho/Cr was elevated ($p < 0.01$, $d = 0.44$), and NAA/Cr was reduced ($p < 0.001$, $d = 0.35$) in the CSF A β 42-positive MCI group.

More importantly, mI/Cr was significantly increased in CSF A β 42-positive healthy controls compared to CSF A β 42-negative healthy controls ($p < 0.05$, $d = 0.46$), revealing that mI/Cr levels are already changed in PCC/precuneus of asymptomatic individuals at risk for developing AD.

MRS and CSF biomarkers. In the entire cohort, we found that decreased CSF A β 42 was associated with increased mI/Cr ($\hat{\beta} = -0.23$, $p < 0.001$) as well as decreased NAA/Cr ($\hat{\beta} = 0.11$, $p = 0.05$). Further, elevated CSF tau levels correlated with decreased NAA/Cr levels ($\hat{\beta} = -0.14$, $p = 0.01$).

In the cognitively healthy group, we found that increased mI/Cr correlated with decreased CSF A β 42 ($\hat{\beta} = -0.21$, $p = 0.002$), confirming that mI levels are associated with amyloid pathology already during asymptomatic stages.

Finally, in the CSF A β 42-positive MCI group, we detected a significant association between NAA/Cr and CSF t-tau. Both measures are known correlates of the extent of neuronal injury and degradation—a process ongoing in patients with MCI.

See table e-4 for a complete account of significant associations between MRS and CSF markers.

MRS and [¹⁸F]-flutemetamol PET. CSF A β 42 and amyloid PET reflect somewhat different aspects of A β pathology.²¹ Therefore, we explored whether MRS metabolites were associated with cortical retention of the amyloid PET ligand [¹⁸F]-flutemetamol in predementia AD.

We found that increased level of mI/Cr was linked to higher [¹⁸F]-flutemetamol retention in the PCC/precuneus region in healthy elderly individuals. Further, this association was only present in the CSF A β 42-positive healthy controls (table 2 and figure 2), and was absent in the CSF A β 42-negative healthy controls.

The relationship between mI/Cr and amyloid plaque load in amyloid-positive healthy controls was sustained when amyloid positivity was based on [¹⁸F]-flutemetamol retention rather than CSF A β 42 levels (table 2 and appendix e-2). Further, when [¹⁸F]-flutemetamol retention in the PCC/precuneus was used as a proxy for local A β pathology, elevated Cho/Cr was linked to an increased plaque load in PET-positive controls (table 2).

Table 2 Significant associations between brain metabolites measured by magnetic resonance spectroscopy and [¹⁸F]-flutemetamol uptake in the posterior cingulate cortex/precuneus in healthy controls

	Predictor PET Aβ parameters		
	Standardized beta β	T	p Value
All healthy controls			
ml/Cr	0.32	3.45	<0.001
CSF Aβ42-positive controls			
ml/Cr	0.44	2.62	0.02
Amyloid PET-positive controls			
ml/Cr	0.48	2.76	0.01
Cho/Cr	0.44	2.52	0.02

Abbreviations: Cho = choline; Cr = creatine; ml = myo-inositol. Parameters of stepwise multiple linear regression models, where age and sex were used as covariates when the effects of these confounders were significant.

MRS and functional MRI. Next we studied whether MRS metabolites in precuneus/PCC were associated with functional connectivity between precuneus/PCC and other regions within the DMN. We found that increasing levels of ml/Cr, but not NAA/Cr, were associated with reduced DMN connectivity across all groups ($r = -0.16$, $p = 0.02$) (figure 2).

We further investigated whether the association between ml/Cr levels and DMN connectivity was driven by Aβ pathology. Entering CSF Aβ42 as a covariate in a GLM yielded $t = -2.08$ ($p = 0.04$) for ml/Cr and $t = 0.63$ ($p = 0.53$) for CSF Aβ42, suggesting that the relationship between functional connectivity and ml/Cr may be Aβ-independent. Using [¹⁸F]-flutemetamol retention in the PCC/precuneus as a proxy of Aβ pathology gave analogous results (appendix e-2).

MRS and APOE genotype. Finally, we aimed to study the relationship between APOE and MRS metabolites in different stages of prodementia AD. GLM analysis revealed that healthy APOE ε4 carriers with still normal CSF Aβ42 levels had significantly higher ml/Cr concentrations than APOE ε4 noncarriers ($t = -3.61$, $d = 0.47$, $p < 0.001$). APOE ε4 carriership did not influence ml/Cr levels in the remaining diagnostic groups—those where CSF Aβ42 levels were already abnormal (figure 3).

DISCUSSION The present study aims to describe changes in the MRS neurochemical profile across 4 well-characterized groups that follow the hypothetical course of prodementia AD.²² Biomarker-negative participants with no evidence of subtle cognitive decline are plausible true controls unlikely to be at risk for AD. At-risk individuals include those with no cognitive symptoms but with abnormal CSF Aβ42

levels, as well as patients with SCD and MCI with evidence of abnormal CSF Aβ42.

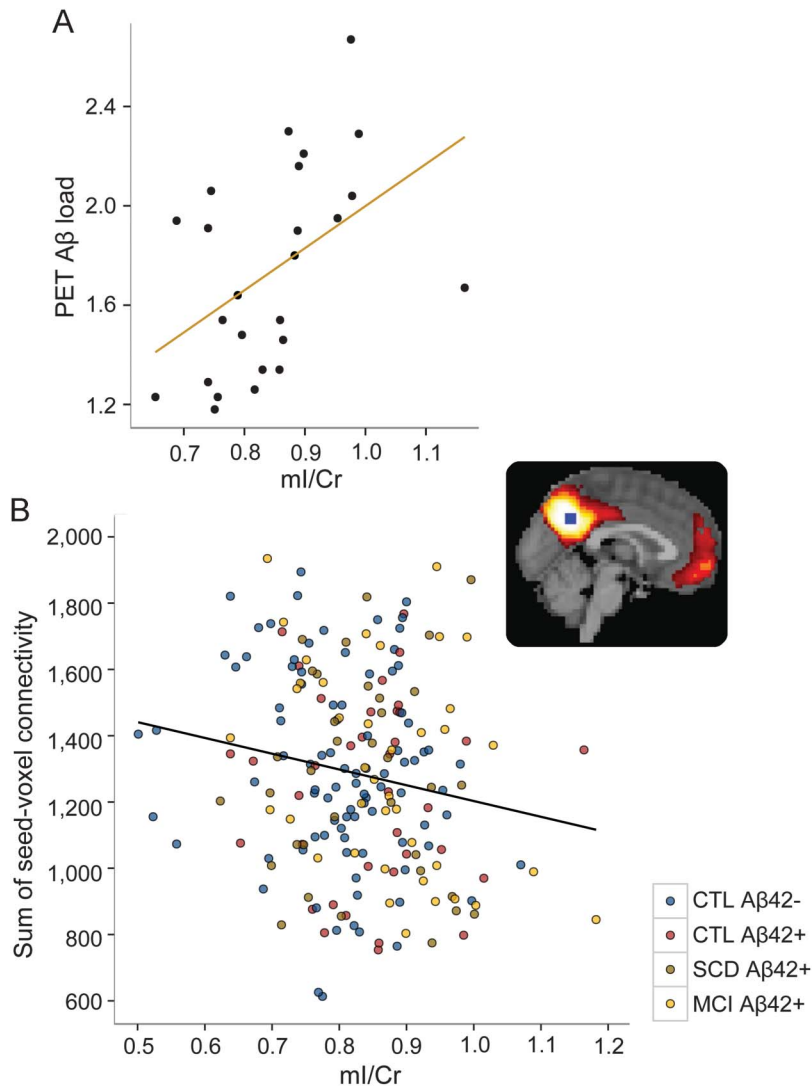
In this study, stratification of participants into groups was based in part on CSF Aβ42 cutoff values. A cutoff might in some cases mask subclinical effects—those that take place below the set threshold. We also used classification cutoffs based on mixture modeling for [¹⁸F]-flutemetamol PET data. Although this method has been extensively used previously to establish unbiased thresholds,²³ such a cutoff is nevertheless study-specific.

Elevated ml/Cr and decreased NAA/Cr belong to the more reproducible MRS findings in AD, with abnormal ml/Cr appearing earlier and decreased NAA/Cr later in the disease course.²⁴ As expected, we detected that NAA/Cr and ml/Cr ratios were significantly different between biomarker-negative controls and biomarker-positive patients with MCI—the 2 groups on the opposite ends of the prodementia spectrum. More intriguingly, we detected that abnormal elevation of ml/Cr in the PCC/precuneus region occurs already in the asymptomatic at-risk individuals.

We found that early changes in ml/Cr were temporally associated with the initial decline in CSF Aβ42 levels, as the increase in ml/Cr was linked to a decrease in CSF Aβ42 in controls, but not in symptomatic individuals. CSF Aβ42 levels are already fully decreased 10–20 years before dementia onset^{25,26}; brain ml/Cr is increased already at prodementia stages in individuals with Down syndrome²⁷ and familial dementias.²⁸ Although the mechanistic linkage between CSF Aβ42 and ml/Cr cannot be established directly from our study, their contemporaneous changes may be related to the same pathologic process, one that predates cognitive decline.

Abnormally low levels of CSF Aβ42 are accurate in revealing disturbed Aβ metabolism. Although CSF Aβ42 predicts conversion to dementia, levels do not correlate well with rate of disease progression. In contrast, the amount of cortical retention of amyloid PET ligands is more directly related to the accumulation of Aβ fibrils in the neocortex, increasing continuously throughout the prodromal stages of AD.²⁹ In the largest study linking changes in MRS measures to in vivo Aβ deposition,¹¹ cognitively normal elderly participants demonstrated significant positive association between ml/Cr measured in the PCC and cortical retention of Pittsburgh compound B. We detected an analogous association between ml and plaque load in healthy controls as ml/Cr concentrations were shown to correlate with [¹⁸F]-flutemetamol uptake. Moreover, we detected that this association was driven by the subgroup harboring amyloid burden, and was absent in amyloid-free participants.

Figure 2 Associations between myo-inositol (mI)/creatine (Cr) and A β plaque load and functional connectivity



(A) Association between mI/Cr in posterior cingulate cortex (PCC)/precuneus and A β plaque load measured by [18 F]-flutemetamol PET in CSF A β 42-positive controls: a significant association between mI/Cr and plaque load ($\beta = 0.42$, $t = 2.62$, $p = 0.02$). (B) Association between mI/Cr in PCC/precuneus and functional connectivity. The sum of each participant's seed-voxel correlation within a normal connectivity mask correlated significantly with mI/Cr levels in the seed region across participants from all groups, $r_{\text{pearson}} = -0.16$ ($p = 0.02$). The inset shows the seed position in precuneus (in blue) and the thresholded average normal connectivity of CSF A β 42-negative controls used to define the normal connectivity mask. CTL A β 42 $-$ = controls with CSF A β 42 >530 ng/L; CTL A β 42 $+$ = controls with CSF A β 42 \leq 530 ng/L; SCD A β 42 $+$ = patients with subjective cognitive decline with CSF A β 42 \leq 530 ng/L; MCI A β 42 $+$ = patients with mild cognitive impairment with CSF A β 42 \leq 530 ng/L.

Regarding MRS measures and CSF neurodegenerative markers, we detected that an increase in NAA/Cr was linked to a decrease in CSF t-tau in the CSF A β 42-positive MCI group. Both measures are markers of neuronal damage; an association between the 2 in prodromal MCI only is consistent with the fact that neuronal injury takes place at a later time point in the prodementia continuum. Loss of NAA/Cr in the PCC/precuneus at the

symptomatic stage preceding AD is in line with this region's involvement in attention and memory.³⁰

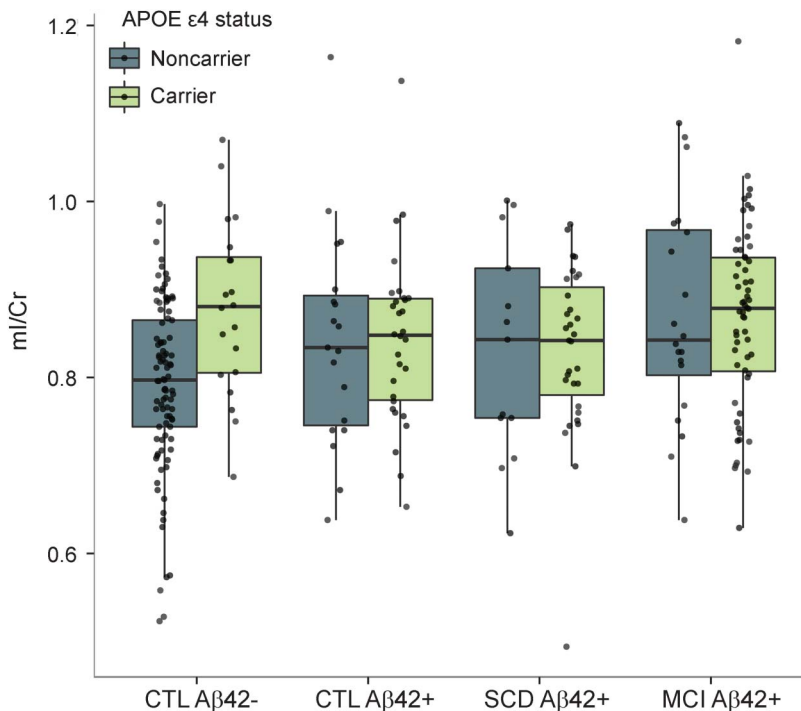
The significance of the changes in Cho/Cr in AD is not well-understood. We detected elevated Cho/Cr levels in CSF A β 42-positive MCI compared to CSF A β 42-negative controls—a finding often attributed to an upsurge in membrane turnover due to neurodegeneration, or possibly underlying cholinergic dysfunction in the MCI group.¹¹

Carriership of the *APOE* ϵ 4 is the main genetic risk factor for AD. *APOE* ϵ 4 enhances AD pathology, and is thought to trigger the initiation and acceleration of A β deposition in the brain,³¹ without having any primary effect on A β production.³² *APOE* ϵ 4 also contributes to AD pathogenesis by being implicated in the process of neuroinflammation.³¹ Regarding the relationship between *APOE* genotype and MRS measures, elevated mI/Cr has previously been detected in the PCC/precuneus of older ϵ 4 carriers compared to noncarriers in a healthy aging population.¹² However, whether this was a consequence of underlying A β pathology in ϵ 4 carriers has not been investigated previously. We show that in cognitively healthy amyloid-free elderly, mI/Cr levels were significantly elevated in the ϵ 4 carriers compared to the noncarriers. In the remaining subgroups—those with evidence of A β pathology—mI/Cr levels were no longer modified by *APOE* carriership. It seems that mI/Cr has the potential to detect manifestations of the *APOE* genetic effect, which precede cognitive decline and may antedate or be independent of amyloid pathology, perhaps even detecting the more pronounced proinflammatory state associated with ϵ 4 carriership.

The PCC/precuneus region plays a key role within the DMN, operating as a node for structural and functional connections.³⁰ Regions involved in the DMN are affected by amyloid deposition early in the course of AD.³³ We observed a hitherto unreported association between elevated mI/Cr levels and reduced DMN connectivity. Furthermore, our findings suggest that the link between mI/Cr in the PCC/precuneus and functional connectivity in this region may not be a direct consequence of A β deposition.

Increasing attention is being paid to detailed characterization of the earliest stages of AD, warranting further research into cost-effective noninvasive early markers. Although the exact mechanisms behind early changes in the MRS profile cannot be inferred directly from the results of this study, our findings situate changes in the MRS profile within the context of existing clinical, pathologic, and functional information in individuals at risk for AD. We provide evidence of the involvement of brain mI at very early stages in AD progression; in particular our data show

Figure 3 Concentrations of myo-inositol (mI)/creatine (Cr) in posterior cingulate cortex/precuneus in the diagnostic subgroups stratified by APOE $\epsilon 4$ carriership



Levels of mI/Cr differed significantly between carriers and noncarriers in the subgroup of CSF A β 42-negative controls ($t = -3.61$, $p < 0.001$). CTL A β 42 $^-$ = controls with CSF A β 42 >530 ng/L; CTL A β 42 $^+$ = controls with CSF A β 42 ≤ 530 ng/L; SCD A β 42 $^+$ = patients with subjective cognitive decline with CSF A β 42 ≤ 530 ng/L; MCI A β 42 $^+$ = patients with mild cognitive impairment with CSF A β 42 ≤ 530 ng/L.

that mI/Cr levels are elevated in APOE $\epsilon 4$ carriers with no evidence of amyloid pathology.

AUTHOR CONTRIBUTIONS

Study design and concept: O. Voevodskaya and Drs. Westman and Hansson. Acquisition, analysis, and interpretation of data: O. Voevodskaya and Drs. Sundgren, Strandberg, Zetterberg, Westman, and Hansson. Drafting of the manuscript: O. Voevodskaya and Drs. Westman and Hansson. Critical revision of the manuscript for important intellectual content: Drs. Sundgren, Strandberg, Zetterberg, Minthon, Blennow, and Wahlund. Statistical analysis: O. Voevodskaya and Dr. Strandberg. Obtained funding: Dr. Hansson. Administrative, technical, and material support: Drs. Sundgren, Strandberg, Zetterberg, Minthon, Blennow, and Wahlund. Study supervision: Drs. Westman and Hansson.

ACKNOWLEDGMENT

The authors thank the collaborators of this study and the BioFINDER Study group (www.biofinder.se), including Susanna Vestberg for classifying the MCI-AD patients into MCI subgroups, Erik Stomrud and Katarina Nägga for clinical evaluations of cognitively healthy individuals, Sebastian Palmquist for clinical evaluations of patients with mild cognitive symptoms, Per Wollmer for help with [18 F]-flutemetamol PET imaging, Olof Lindberg for volumetric measurements, and Peter Mannfolk for resting-state fMRI data.

STUDY FUNDING

Work in the authors' laboratory was supported by the European Research Council, Swedish Research Council, the Crafoord Foundation, the Swedish Brain Foundation, the Skåne University Hospital Foundation, the Swedish

Alzheimer Association, the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson's disease) at Lund University, Swedish Brain Power, and the Strategic Research Programme in Neuroscience at Karolinska Institutet (StratNeuro). The study was funded by The Torsten Söderberg Foundation (Dr. Blennow) and the Swedish federal government under the ALF agreement. Doses of [18 F]-flutemetamol were provided by GE Healthcare.

DISCLOSURE

O. Voevodskaya, P. Sundgren, O. Strandberg, H. Zetterberg, and L. Minthon report no disclosures relevant to the manuscript. K. Blennow has served on advisory boards for Eli-Lilly, IBL International, Novartis, and Roche Diagnostics. L. Wahlund, E. Westman, and O. Hansson report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received September 4, 2015. Accepted in final form January 14, 2016.

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