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Kathrin Muth

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Mild cognitive impairment in the elderly is associated with volume loss of the cholinergic basal forebrain region

Kathrin Muth[1], Ralf Schönmeyer[1,2], Silke Matura[1], Corinna Haenschel[1,2], Johannes Schröder[3], Johannes Pantel[1*]

[1] Dpt. of Psychiatry, Psychosomatics and Psychotherapy, Hospital of the Goethe-University, Frankfurt a.M., Germany

[2] Max-Planck Institute for Brain Research, Frankfurt a.M., Germany

[3] Dpt. of Geriatric Psychiatry, Heidelberg University Hospital, Heidelberg, Germany

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*** Corresponding Author:**
Prof. Dr. Johannes Pantel
Chair of Geriatric Psychiatry
Hospital of the Goethe-University
Heinrich-Hoffmann-Strasse 10
60528 Frankfurt a.M., Germany
e-mail: johannes.pantel@kgu.de
phone: +49-69-6301-7094
fax: +49-69-6301-5189

Abstract

Background: Cholinergic neurons within the basal forebrain are assumed to be an early (preclinical) manifestation site of pathological changes in Alzheimer's disease (AD). **Aim:** We used morphometric MRI to detect and quantify atrophic changes in the basal forebrain of subjects suffering from amnestic mild cognitive impairment (aMCI). **Method:** 3 Tesla MR data of 26 aMCI patients, 46 cognitively normal elderly controls (CO), and 12 patients suffering from Alzheimer's dementia were analyzed, including segmentation and quantification of brain tissue as well as a segmentation of basal forebrain structures (substantia innominata, SI). **Results:** We found the volume of the SI to be significantly different between groups, in that control subjects showed the largest SI-volumes, followed by aMCI and AD-patients. **Conclusion:** These results are in line with the hypothesis that cell loss within the cholinergic basal forebrain regions occurs already in the early (predementia) stage of AD. Invivo quantification of these changes might be of use as a novel neuroimaging marker of cholinergic neurodegeneration in AD.

Introduction

Cholinergic dysfunction plays an important role in the cognitive impairment associated with Alzheimer's disease (AD) (1). The magnocellular neurons of the nucleus basalis Meynert located in the substantia innominata (SI) of the basal forebrain are the main source of cholinergic projections to the cerebral cortex. Accordingly, previous MRI studies indicated atrophic changes of the basal forebrain in patients with clinically diagnosed AD (2, 3). Recent post-mortem studies demonstrated that pathological alterations of the cholinergic nuclei within the basal forebrain are already present in mild cognitive impairment (MCI), the putative transitional stage between normal aging and dementia (4, 5). While quantitative MRI has been widely used to investigate atrophy of the medial temporal lobe structures in MCI (6), little is kown about its feasibility to detect early SI changes in the predementia stages of AD. In this study we used in vivo morphometric methods to measure the volume of the SI in patients with MCI compared to AD-patients and cognitively normal elderly controls. We tested the hypothesis that MCI is characterized by volume loss of the SI which is detectable by MRI.

Methods. Twenty-six patients with aMCI (modified Petersen criteria) (7) were compared with 46 elderly control subjects and 12 AD-patients (NINCDS-ADRDA criteria) (Table1). All subjects underwent neuropsychological testing (Consortium to Establish a Registry for Alzheimer`s Disease (CERAD)) (8) to control for cognitive impairment, especially memory dysfunction. Only patients with amnestic MCI (with and without dysfunctions in additional cognitive domains) were included. In addition to clinical and neuropsychological investigations all participants were genotyped for APOE allele type using a standard polymerase chain reaction-based method. All MR examinations were performed on the same 3.0 Tesla scanner (Trio; Siemens Medical System, Erlangen, Germany). The examination protocol included a high resolution T2-weighted sequence to image the SI-area using the following parameters: TR (repetition time) = 5000 ms, TE (echo time) = 137 ms, slice thickness = 1 mm, voxelsize = 0.5 x 0.5 x 1.0 mm), FOV field of view) = 256 mm, base resolution = 512. Additionally we performed a T1-weighted MPRAGE-Sequence (TR = 2300 ms, TE = 3.93 ms, slice thickness = 1 mm, FOV = 256 mm, base resolution = 256) which was used for tissue segmentation and evaluation of global brain volume.

The SI can be visualized as a narrow band below the globus pallidus and above the CSF contrast (Figure1). The structure-volume of the SI on both sides was measured on three coronar slices, 1mm before the anterior commisure (AC), through AC and 1 mm beyond AC. Relevant areas on each slice were manually traced, using a custom made segmentation program (vmr segmenter v0.8 by R. Schoenmeyer©) (9). Raters were blinded to group membership of the participants dataset. Interrater reliability was established to be r = 0.82 using an independent dataset. Detailed tracing guidelines including description of neuroanatomical landmarks can be provided by the authors on request. In addition to manual tracing, segmentation of the entire MR data sets into white matter (WM), gray matter (GM), and cerebrospinal fluid (CSF) was performed using the SPM5 package (http://www.fil.ion.ucl.ac.uk/spm/). Intracranial volume (ICV) was calculated by adding all three compartment volumes and used as a covariate for the statistical analysis. Statistical analyses was done with SPSS 15.0 (SPSS Inc., Chicago, Ill., USA). The study was approved by the ethics committee of the University Hospital Frankfurt a.M.

Table 1: Groups characteristic

	CO	**aMCI**	**AD**	**p**
N	46	26	12	
Age (y)	63.4 (6.4)	67.6 (6.7)	72.6 (6.6)	<.001 [a]
Sex (f/m)	27/19	14/12	6/6	n.s. [a]
Education (y)	13.6 (2.4)	13.2 (2.5)	12.9 (2.7)	n.s. [a]
MMSE score	29.5 (0.8)	27.5 (1.8)	24.1 (4.2)	<.001 [a]
APOE ε4 carrier (%)	19.5	33.3	50.0	<.001 [b]
Whole brain GM (%)	40.15	38.52	37.17	<.002 [a]
Whole brain WM (%)	30.38	29.55	27.79	<.005 [a]

CO, Control subjects; aMCI amnestic mild cognitive impairment;
AD, Alzheimer´s Disease; MMSE, Minimental State Examination;
APOE, Apolipoprotein E; a, ANOVA; b, $\chi 2$ test; n.s., not significant;
GM, Grey matter, WM, White matter; values are means (+- standard deviation).

Results. The volume of the SI was significantly different between groups (ANCOVA, $p < 0.001$, F=11.38, df=4 using age and ICV as covariates, Bonferroni corrected; ANOVA F=32.22, df=2, Tukey test for multiple comparisons: controls vs. MCI $p< 0.01$, controls vs. *AD* $p < 0.001$, MCI vs. AD $p < 0.05$). In control subjects SI-extent was the largest (0.25 ± 0.065 ccm), followed by a considerable loss of substance in the MCI group (0.19 ± 0.050 ccm; -24% compared to controls) and a pronounced atrophy of the SI in the AD group (0.12 ± 0.032 ccm; -52% compared to controls). In comparison to the SI-volume loss, global brain volume (GM, WM) in MCI and AD was less atrophied (Table 1) when compared between groups. (ANOVA, Tukey test for multiple comparisons; controls vs. AD, GM: F=6.90, df=2, p<.002; controls vs. AD WM: F=5.71, df=2, p<.002) . Data shown in figure 2 are mean values of both hemispheres. There was no difference between left and right SI volume for all groups. ApoE ε4 allele frequency (number of ε4 allele) differed ($\chi^2 = 20.19, p = 0.0004$) between groups: 50% of the AD-patients were ε4 positive. In MCI patients the frequency of ε4 was 33.3% and control subjects only exhibited the ε4 allele in 19.5% of all cases. There were no within group differences of SI volume related to the presence or absence of the ε4 allele.

Exploratory testing for any relationship between cognitive function and volume of the SI revealed a correlation between performance in the word list recall task (CERAD subtest) and SI-volume in AD patients (r^2=0.487, p<.037).

Discussion. Degeneration of basal forebrain areas including the SI is a key event in the pathogenesis of AD. Accordingly, previous MRI studies reported atrophy of these areas in AD patients compared to elderly control subjects using one-dimensional measures of thickness (5) as well as voxel-based morphometry (VBM) (3). Hitherto there is only little evidence on measurable substance loss of the basal forebrain in the predementia stages of AD. Based on neuropathological findings it might be speculated that tissue loss of the SI can already be detected and quantified in patients with MCI (4, 5, 10, 11,12). These individulas are at an increased risk to develop AD and thus represent an important target group for (secondary) intervention measures for the prevention of dementia.

Recently, Hall et al. (13) used VBM to identify atrophy of the medial basal forebrain in a subgroup of 21 cognitively healthy elderly participants of the Cardiovascular Health Cognition study who developed dementia at follow-up. In line with these findings our study for the first time demonstrates that volumetric MRI is a feasible tool to detect volume loss of the SI in vivo in a clinically characterized risk group for the development of AD (as defined be the aMCI concept). Our results are consistent with the hypothesis that there is already cell damage and neurodegeneration in the cholinergic basal forebrain region in subjects suffering from MCI. The presented findings are further in line with recent histological studies showing cytopathological changes in basal forebrain cholinergic neurons to be present very early in the aging-MCI-AD continuum (11). The recently published data of Zaborszky et al. (14) on probabilistic maps of the different magnocellular basal forebrain cell groups are of special interest to investigate distinct neurodegenerative processes within the SI-basal forebrain area using MRI.

A further question addressed in our study was the influence of APOE ε4 status on volume loss in the SI. The APOE ε4 allele is a known risk factor for AD and the results of clinical trials using cholinergic substances suggest an influence of APOE ε4 status on therapeutic effects in AD and MCI (15, 16). Previous MRI studies found increased atrophy of medial temporal lobe regions in MCI patients positive for APOE ε4 (17). Similarly we suggested an influence of the genotype on SI volume between groups. So far we did not prove this relationship for the

basal forebrain region neither in patient groups (AD, MCI) nor in cognitively unimpaired elderly controls. However, since sample size is one limiting factor of our study this might explain these negative findings.

In contrast to other morphometric studies on SI size which measured the thickness of the structure on a discrete slice, we used a volumetric approach to capture a larger portion of the SI. Furthermore, our method is characterized by its high neuroanatomical validity and should be less susceptible to post processing artifacts (which might easily be introduced when using automated measures such as VBM; 3,13). Our approach allowed to reliably quantify the volume loss of the SI in the diagnostic groups. The cross-sectional analysis revealed it to be –24% in aMCI and –52% in the group of mildly demented AD patients (MMSE: 24.1 $\pm$4.2). This volume loss is considerably higher than values reported for medial temporal lobe atrophy in MCI and mild AD (6,18) and also much higher than our findings for global brain atrophy (Table 1). These results refer to the basal forebrain region as an early neuronal correlate of degenerative processes in the CO-MCI-AD continuum. Accordingly, prospective longitudinal studies should clarify if our method can be used as a sensitive diagnostic marker of early (predementia) AD. In this context it is of special interest to clarify which area is the first to undergo pathological changes in terms of degeneration and MR detectable volume loss as well as to address potential correlations between neurodegeneration in basal forebrain structures and cortical volume loss in regions receiving cholinergic input. Based on our findings it would also be worthwhile to investigate the validity of SI atrophy as a predictor of therapeutic response in trials using cholinergic antidementia drugs or as a surrogate marker for the monitoring of cholinergic neurodegeneration during the course of the disease.

Kathrin Muth

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Figure legends:

Figure 1. MR Imaging of the Substantia Innominata (SI). (A) Slice-group covering the SI, perpendicular to a plane through the anterior and posterior commisure. (B) coronar slice through the anterior commisure. (C) Comparison of SI-atrophy (arrow) between groups.

Figure 2. Boxplot of the Substantia Innominata Volume in patients with AD, MCI- and control subjects. The central box represents values from the 25th to the 75th percentiles; the middle line represents the median, and the horizontal line extends from the minimum to the maximum value. The median SI volume is lowest in the AD group compared with MCI and control subjects. CO, Control subjects; MCI, amnestic mild cognitive impairment; AD, Alzheimer´s Disease. Stars (*) indicate significant differences between groups; * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$.