Annexure-IX

PROJECT COMPLETION REPORT

Notes: 1. 10 copies of the Project Completion Report (PCR) should be sent within one month of the completion or termination of the project. 2. The PCR should be in bound form. 3. Cover page should include the title of the project, file number, names and addresses of the investigation.

1. Title of the project:

The Human Brain Mapping Project- A resting state fMRI study of healthy controls and patients with Mild Cognitive Impairment (MCI) and Degenerative dementia of the Alzheimer's type.

2. Principal Investigator(s) and Co-Investigator(s):

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3. Implementing Institution(s) and other collaborating Institution(s):

Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram

4. Date of commencement: 13/10/2014

5. Planned date of completion: 22/09/2018 (extension period)

6. Actual date of completion: 22/09/2018

7. Objectives as stated in the project proposal:

   i. Identification of resting state networks in the normal human brain via functional and structural connectivity analysis (independent-component analysis) and hence examine the structural connectivity of individual resting state networks (RSN) with spatial correlation using diffusion tensor imaging (DTI) and hence derive a large scale network (LSN) analysis.

   ii. Comparison of large-scale network analysis in age-matched cognitively normal healthy controls (CNHC), patients with mild cognitive impairment (MCI) and patients with early Alzheimer's Dementia (AD).

8. Deviation made from original objectives if any, while implementing the project and reasons thereof: Nil

9. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:

The project started on 13/10/2014 after approval from the SCTIMST - Institute Ethics Committee and receipt of funds from DST.

In this study, 135 subjects were prospectively recruited from a Memory and Neurobehavioral Disorders Clinic of a reputed hospital in the South Indian state of Kerala. These included 41 controls; 54 MCI patients and 40 AD patients. All subjects provided written consent according to procedures approved by the Institutional Ethics Committee of our institute. Participants with Mini–Mental State Examination (MMSE) score between 28 and 30, a 0 score of clinical dementia rating (CDR) with formal education of >10 years, no subjective memory complaints, and with no serious neurological/psychiatric issues were selected as healthy controls. Participants with CDR of<2 and MMSE score between 13 and 21 were confirmed as AD according to standard NINCDS–ADRDA diagnostic criteria and MCI patients were diagnosed as per the modified Petersen's criteria with CDR of ≤0.5 and MMSE score between 24 and 29. The participants were cognitively screened by the vernacular adaptation of Addenbrooke's Cognitive Examination battery (ACE) and other area specific neuropsychological tests as validated before.

Sequence parameters used in this study were: Structural images were obtained using a high resolution reference axial 3D brain volume imaging sequence (3D Bravo) with TR/TE = 7/2.98 ms, slice thickness = 1 mm, flip angle = 12°, matrix size = 256 × 256, and voxel size = 1 mm × 1

mm × 1 mm. Resting fmri were acquired with TR/TE = 2500/30, flip angle = 80°, NEX = 1, slice thickness = 3.2. DTI was acquired with TR.TE = 8975/101.8, slice thickness = 2.0, flip angle = 90°. MRS were acquired with TR/TE = 1000/35.0, slice thickness = 15.0, NEX = 2. Further, ASL images were acquired by employing 3D fast spin echo pseudo-continuous ASL sequence with acquisition parameters: TR/TE = 4852/10.70 ms, flip angle = 111°, voxel size = 1.875 x 1.875 x 4, slice thickness = 4 mm, NEX = 3 and post label delay = 2025 ms.

All participants were informed to lie down with their eyes closed in the scanner.

10. Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:

- In the first phase, a pilot study was conducted in 1.5 T scanners to standardize the protocol. A total of 12 dementia subjects and 10 controls underwent MRI with the specified protocol. The collected data were analysed and identified 3 important networks such as default mode (DMN), Executive, Salience, among these, DMN and Salience only showed significant functional connectivity changes in AD patients compared to controls. The functional connectivity measures of DMN were examined using CONN software. The study revealed significant functional connectivity in the DMN of control and not in AD and MCI.
- The structural connectivity analysis failed to reveal any significant change in the WM structural integrity in MCI/AD patients
- A total of 23 MCI, 20 AD subjects and 18 controls underwent 3 Tesla MRI with the specified protocol as mentioned above.
- The collected data were analysed and identified 8 important networks such as default mode (DMN), Executive, Salience, fronto- parietal, Sensory motor, Visual, and Auditory and language network.
- No significant differences were noted in structural and functional connectivity between MCI and controls ($P<0.05$, FWE corrected). The early AD group compared to controls and MCI revealed decreased functional connectivity changes in default-mode network precuneus, supracalcarine cortex) with reduced anterior connectivity and in visual networks (precuneus; lateral occipital cortex; lingual gyrus).
- The structural connectivity analysis failed to reveal any significant change in the WM structural integrity in MCI patients. While, the whole brain TBSS analysis found significant structural changes in forceps minor, forceps major, superior longitudinal

fasciculus, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus in AD compared to controls.

- VBM analysis showed reduced gray matter(GM) density in the right fusiform gyrus, left inferior temporal gyrus, and Middle frontal gyrus in MCI whereas, the AD subjects revealed significant GM reduction in hippocampus, para hippocampus, amygdala, bilaterally, left fusiform gyrus, left inferior temporal gyrus and Middle orbital frontal gyrus compared to controls.
- ROI to ROI whole brain functional connectivity analysis in MCI and AD revealed significantly reduced functional connectivity in interlobe connections such as from temporal to frontal and from temporo-parietal to basal ganglia regions
- Compared to MCI, AD group exhibited reduced connections from posterior cingulate to precuneus.
- Fundamental network parameters of networks: global efficiency, local efficiency, betweenness centrality, cost, clustering coefficient, and degree was lower in MCI and AD compared to controls.
- This less number of control population was not enough to draw a possible conclusion as we have stated. Even though, we found significant structural and functional changes in AD patients compared to controls, we could not find any significant connectivity changes in our MCI cohort and controls. Previous studies with same line of research (Li et al 2016, Zhou et al 2015) demonstrated functional changes in MCI, which might serve as early indicators for the dysfunction and progression in the early stage of AD. They established the intra- and inter-network connectivity changes in around 35 patients and 35 controls. As our cohort is comparatively less, we failed to observe the disrupted interaction in large scale networks.
- Hence, the project was extended for one year to achieve a proper result as per our hypothesis. In the extension phase, we acquired 23 more controls, 31 MCI and 20 AD, making a total of 41 controls, 54 MCI and 40 AD patients.

The results obtained for different multi-modality analysis along with the extension phase are as follows:

I. Resting state fMRI - Results

- In this study, ICA analysis using gift toolbox was performed to examine different temporally coherent networks. 11 resting state networks such as posterior default mode

network, anterior default mode network, precuneus, anterior salience, insula salience, cerebellum, motor, primary medial visual, left parietal, right parietal and sensory motor network were identified (fig. 1). The differences between subject groups were estimated by means of Voxel-wise analysis of variance (ANOVA). The following contrasts were performed: HC>MCI [1 -1 0], HC<MCI [-1 1 0], HC >AD [1 0 -1], HC <AD [-1 0 1], MCI >AD [0 1 -1], MCI<AD [0 -1 1]. The resulting parametric maps of t values were thresholded at $P < 0.05$ uncorrected.

- Further, seed analysis was performed using CONN 17.0 toolbox to measure the strength of the connectivity of the networks that showed a difference between the groups in ICA analysis.

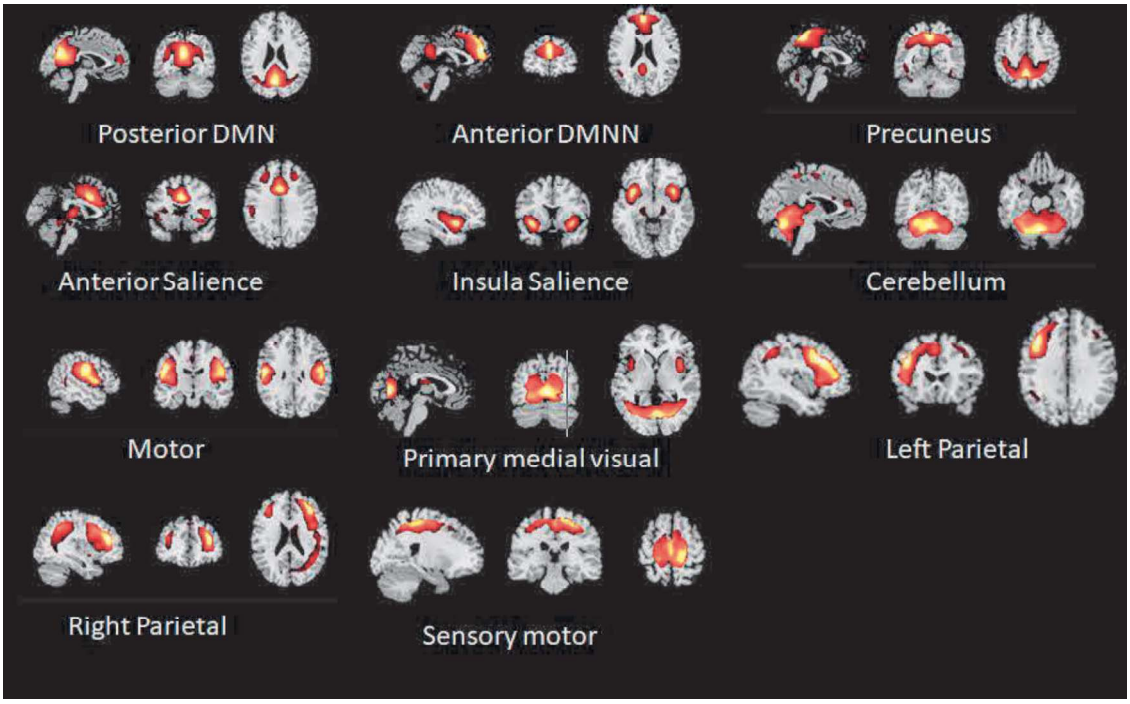

Figure 1 : 11 RSNs identified after ICA analysis

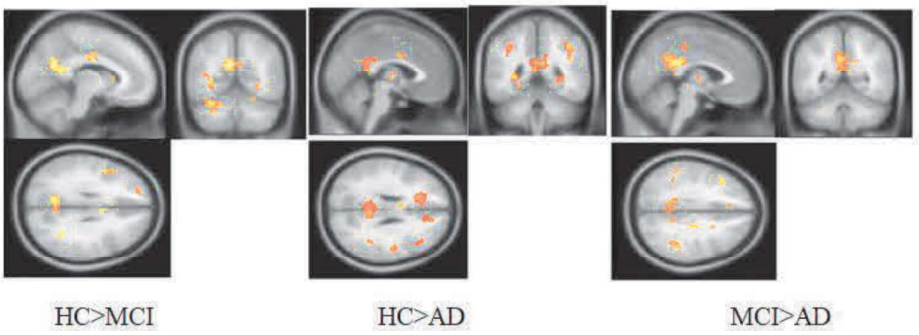

Figure 2 : Group IC maps of Default mode network

Table 1 : Analysis of variance (ANOVA) : DMN variations in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD) compared with cognitively healthy controls (HC) at P<0.05 uncorrected, cluster size threshold: 20

Anatomic Location	Voxel EquivZ	MNI coordinates		
		x	y	z
HC>MCI				
L Precuneus	2.70	-6	-60	24
L Middle Cingulum	2.85	-4	-8	36
L Posterior cingulate	2.43	-26	-72	8
R Putamen	2.12	30	-8	4
R Supramarginal	1.80	54	-34	24
R Fusiform	2.53	24	-72	-8
HC>AD				
L Posterior cingulate	2.29	-2	-42	26
L Frontal superior	3.34	-16	32	34
L Inferior frontal operculum	2.22	-52	10	16
R Supramarginal	3.14	42	-42	40
MCI > AD				
L Postecentral gyrus	2.26	-28	-40	56
L Posterior cingulate	2.81	-4	-40	24
L Medial superior frontal	2.05	-8	30	36
R Posterior cingulate	3.05	4	-44	24
R Middle cingulum	1.70	4	-32	44

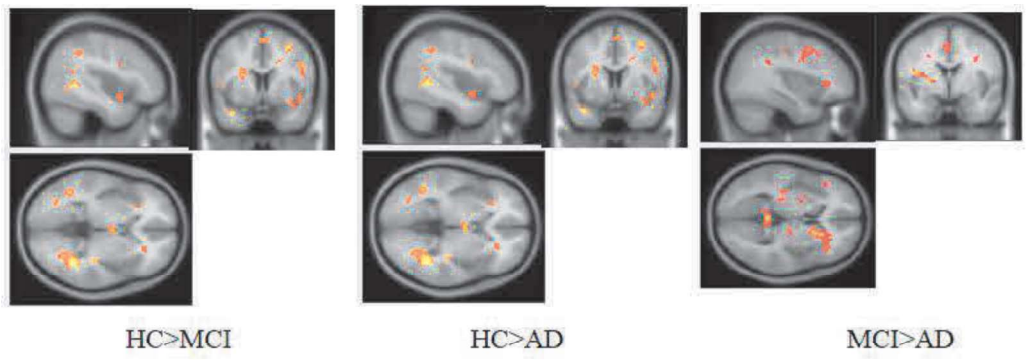

Figure 3 : Group IC maps of Salience network

Table 2 : Analysis of variance (ANOVA) : Salience network variations in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD) compared with cognitively healthy controls (HC) at P<0.05 uncorrected, cluster size threshold: 20

Anatomic Location	Voxel EquivZ	MNI coordinates		
		x	y	z
HC>MCI				
L Insula	2.04	-28	24	4
L Middle frontal	2.63	-36	24	34
L Posterior cingulate	2.43	-26	-72	8
R Inferior frontal operculum	1.83	48	6	22
HC>AD				
L Insula	2.28	-30	6	14
L Frontal superior	3.34	-16	32	34
L Inferior frontal operculum	2.22	-52	10	16
R Supramarginal	3.14	42	-42	40
MCI > AD				
L Insula	2.32	-32	-2	10
L Pallidum	2.25	-20	-2	4
L Rolandic operculum	2.96	-42	-2	14
R Supplementary Motor area	2.73	12	20	46

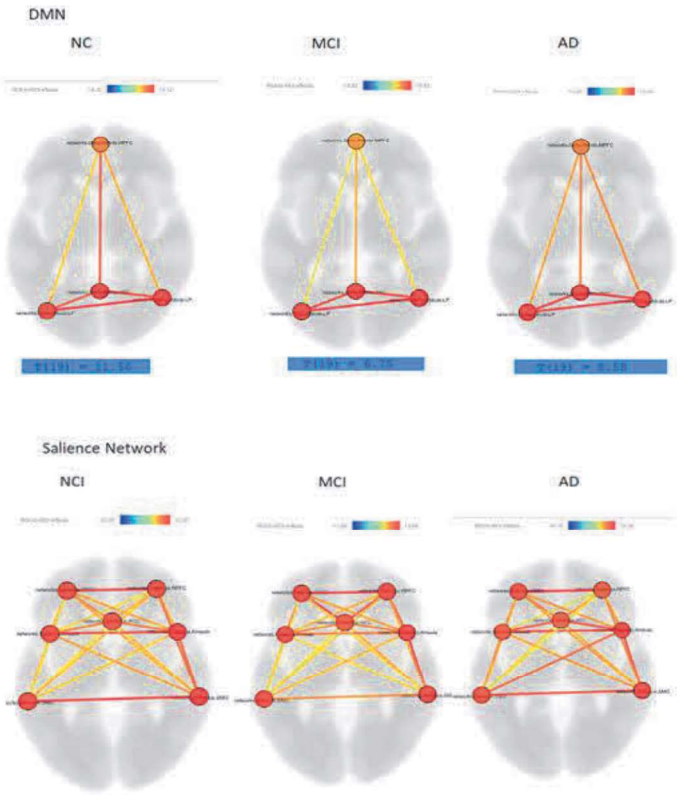

Figure 4: Functional connectivity of DMN and salience network using conn17.0 toolbox

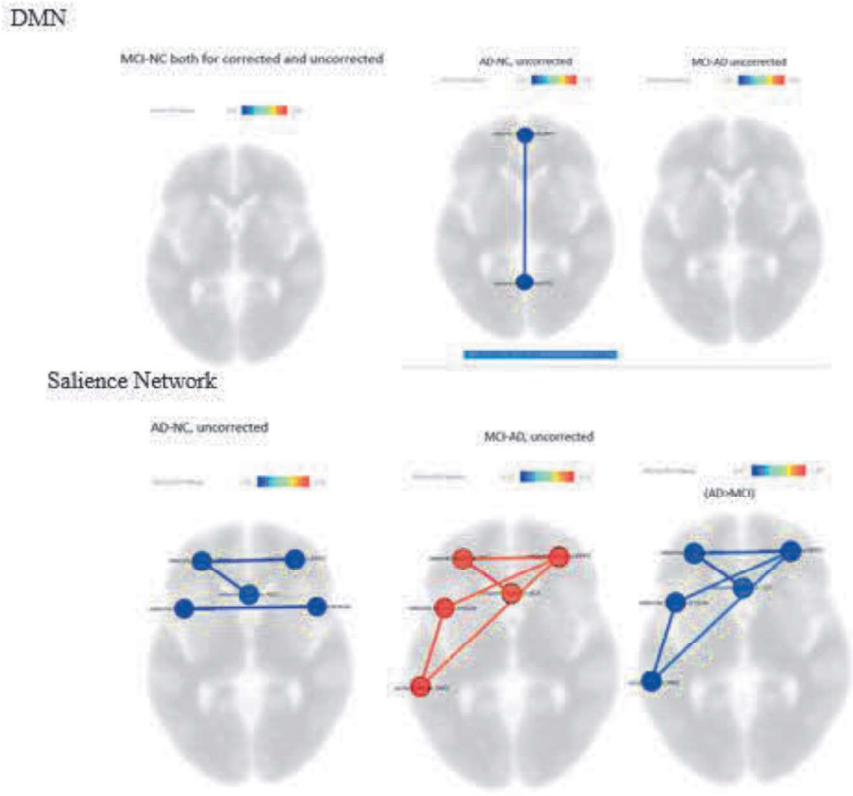

Figure 5: Group difference in functional connectivity in the DMN and Salience networks

II. Volume Based Morphometry (VBM) analysis of the Gray matter

- The gray matter volume differences between subject groups were estimated by means of Voxel-wise analysis of variance (ANOVA) in spm12. Age, gender and total intra-cranial volume (TIV) were used as covariates. The following contrasts were performed: HC>MCI [1 -1 0], HC<MCI [-1 1 0], HC >AD [1 0 -1], HC <AD [-1 0 1], MCI >AD [0 1 -1], MCI<AD [0 -1 1]. The resulting parametric maps of t values were thresholded at P <0.05, corrected for multiple comparisons based on family wise error (FWE).
- Figure 6 shows the significant Gray matter volume alterations revealed by VBM analyses in the three subject groups using the CAT12 toolbox.

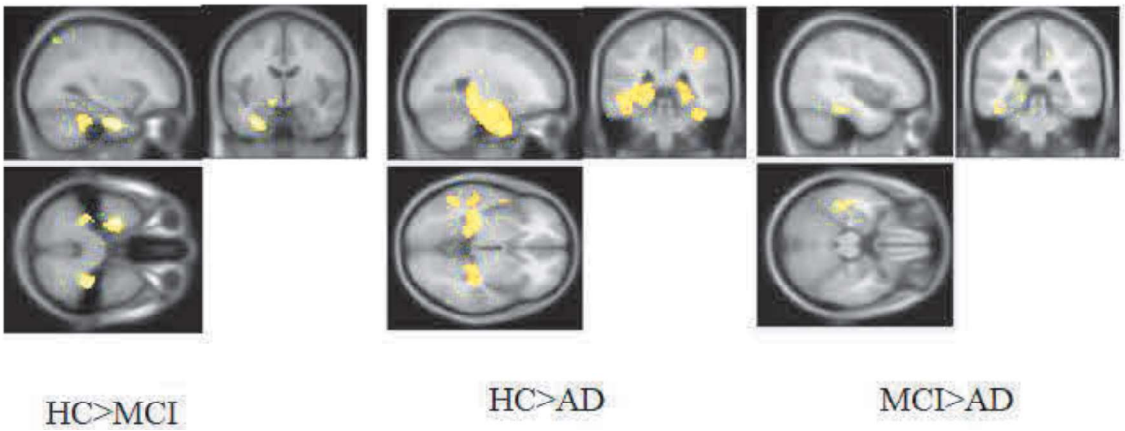

Figure 6: Statistical parametric map showing atrophy patterns in AD and MCI patients

Table 3: Analysis of variance (ANOVA): Gray matter atrophy in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD) compared with cognitively healthy controls (HC) at P<0.05 corrected, cluster size threshold: 20

Anatomic Location	Voxel EquivZ	MNI coordinates		
		x	y	z
HC>MCI				
L Superior temporal	3.54	-48	-9	1.50
L Post central gyrus	3.48	-61.50	-9	37.5
L Parahipocampus	3.78	-24	-10.5	-33
L Fusiform	4.00	-28.5	-9	-37.5
HC>AD				
L Parahippocampus	5.91	-19.5	-6	-33
L Hippocampus	6.11	-24	-6	-22.5
L Amygdala	7.14	-21	-6	-16.50

L Heschl gyrus	5.63	-54	-10.50	7.50
L Middle frontal	6.09	-31.50	49.50	10.50
L Inferior temporal	5.50	-54	-57	-9
R Parahippocampus	5.60	21	-6	-30
R Hippocampus	6.48	22.5	-6	-16.5
R Amygdala	6.13	25.50	-6	-16.50
R Heschl gyrus	5.66	48	-10.50	7.50
R Calcarine	5.75	6	-60	16.50
R Middle occipital	5.81	34.50	-61.50	34.50
R Supramarginal	6.20	37.50	-36	40.50
MCI > AD				
L Inferior temporal	5.38	-43.50	-30	-24
L Parahippocampus	5.53	-19.50	-34.50	-9
L Middle cingulum	5.34	10.50	-34.50	40.50
R Precuneus	5.30	10.50	-67.50	46.50

III. Diffusion Tensor Imaging (DTI) tractography analysis

- DTI analysis was performed on the three subject groups to investigate white matter integrity in AD and MCI using different ROIs for different tracts such as cingulum, forceps major, forceps minor, cortico spinal tract, uncinated fasciculus, superior longitudinal fasciculus, inferior longitudinal fasciculus by means of nordicBrainEx.

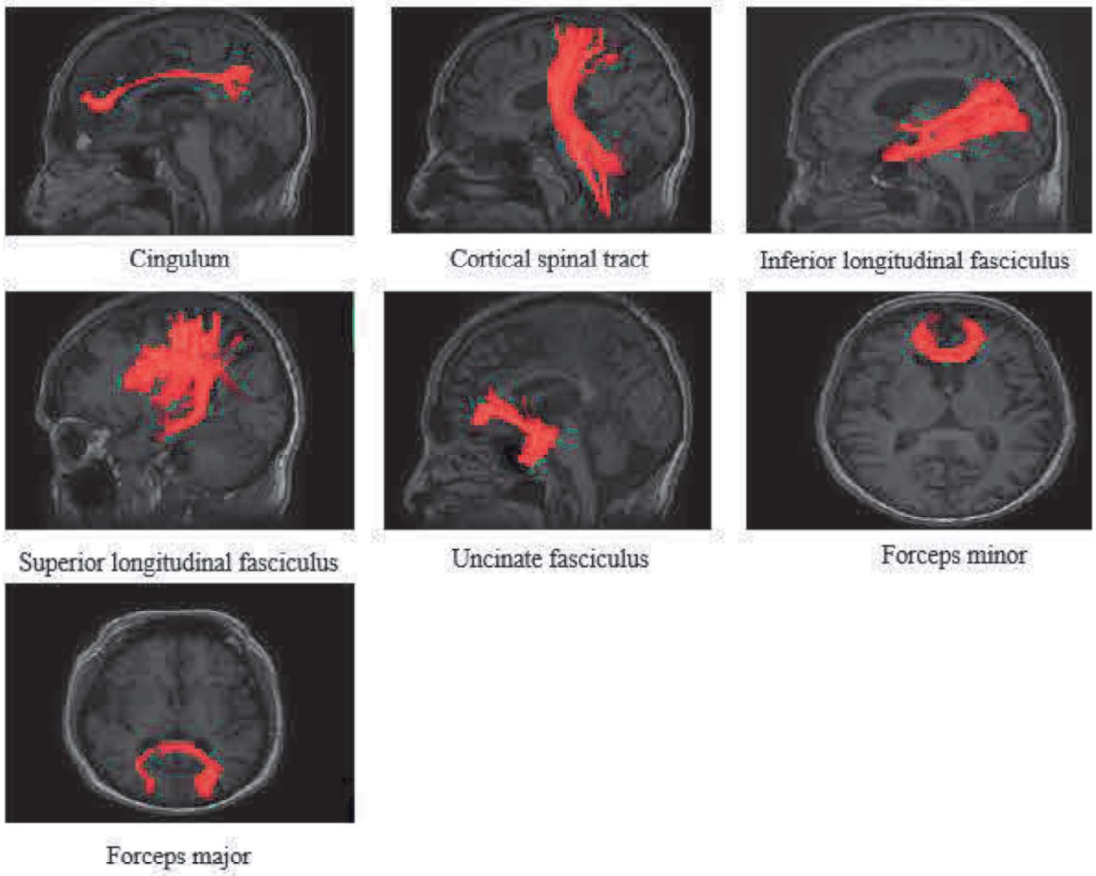

Figure 7: Different DTI tracts obtained for cognitively normal controls

DTI tracts	ADC ($x10^{-3}$ mm^2/s)			FA		
	NC	MCI	AD	NC	MCI	AD
Cingulum	0.754 ± 0.092	0.787 ± 0.097	0.790 ± 0.110	0.56 ± 0.162	0.53 ± 0.13	0.511 ± 0.15
Cortico-spinal tract	0.724 ± 0.143	0.743 ± 0.160	0.740 ± 0.158	0.524 ± 0.18	0.508 ± 0.16	0.588 ± 0.15
Superior Longitudinal Fasciculus	0.702 ± 0.072	0.743 ± 0.095	0.803 ± 0.114	0.501 ± 0.14	0.475 ± 0.131	0.454 ± 0.12
Inferior Longitudinal Fasciculus	0.753 ± 0.132	0.814 ± 0.150	0.875 ± 0.170	0.465 ± 0.135	0.441 ± 0.124	0.437 ± 0.122
Uncinate	0.687 ±	0.730 ±	0.858 ±	0.460 ±	0.451 ±	0.440 ±

	0.103	0.119	0.123	0.181	0.165	0.155
Forceps major	0.721 ± 0.103	0.781 ± 0.188	0.868 ± 0.281	0.601 ± 0.185	0.575 ± 0.177	0.532 ± 0.168
Forceps minor	0.703 ± 0.123	0.721 ± 0.144	0.749 ± 0.185	0.593 ± 0.185	0.575 ± 0.168	0.442 ± 0.149

IV. Arterial Spin Labeling (ASL) analysis

- Second-level statistical procedures implemented in SPM12 was used to statistically analyze the CBF maps and gray matter images. CBF and gray matter volume differences between subject groups were estimated by means of Voxel-wise analysis of variance (ANOVA). Age, gender and total intra-cranial volume (TIV) were used as covariates. The following contrasts were performed: HC>MCI [1 -1 0], HC<MCI [-1 1 0], HC >AD [1 0 -1], HC <AD [-1 0 1], MCI >AD [0 1 -1], MCI<AD [0 -1 1]. The resulting parametric maps of t values were thresholded at $P < 0.001$ uncorrected and at $P < 0.05$, corrected for multiple comparisons based on false discovery rate (FDR).
- Information on regional perfusion values were extracted by means of a region of interest (ROI) analysis. Anatomic ROIs for the regions that showed perfusion changes in the whole brain analysis were defined by means of the WFU Pickatlas tool. Regional CBF was estimated using parameter extraction with Marsbar. Values within ROIs were then averaged for HC, patients with MCI and patients with AD. The regional Gray Matter volume for those regions reported with increased and decreased perfusion were analyzed using estimate ROI mean in cat12 toolbox. The significant areas with atrophy and difference in perfusion were overlaid on T1-weighted standard brain images and the regions were reported in MNI with the help of xjview toolbox.

Table 4: Comparison of demographic and neuropsychological measures between subjects

Characteristic	HC (n = 21)	MCI (n = 20)	AD (n = 19)	Bonferroni corrected P value		
				MCI versus NC	AD versus NC	AD versus MCI
Sex	11/10	11/9	11/8	-	-	-

(male/female)						
Age (mean ± SD in years)	64.57 ± 5.74	66.75 ± 4.08	66.68 ± 5.31	-	-	-
ACE	93.38 ± 4.11	81.12 ± 11.09	72.00 ± 11.09	<0.001	<0.001	0.020
RAVLT cumulative learning score	48.52 ± 8.46	33.47 ± 10.07	25.21 ± 7.15	<0.001	<0.001	0.035
RAVLT 20 min recall score	10.00 ± 2.82	5.41 ± 3.74	1.36 ± 1.73	<0.001	<0.001	0.001

Table 5: Multiple comparison of ROIs for CBF analysis using bonferroni correction among HC, MCI and AD.

ROIS for CBF analysis	HC CBF (ml/100 g/min)	MCI CBF (ml/100 g/min)	AD CBF (ml/100 g/min)	Bonferroni corrected P value		
				MCI versus HC	AD versus HC	AD versus MCI
Superior frontal	39.21 ± 8.16	36.34 ± 7.40	33.81 ± 8.36	0.762	0.111	0.982
Middle frontal	35.20 ± 8.44	32.52 ± 8.79	33.81 ± 8.36	0.956	1.000	1.000
Posterior Cingulate	54.43 ± 10.59	44.26 ± 8.64	39.11 ± 7.72	0.002	<0.001	0.250
Superior temporal	37.21 ± 4.86	35.71 ± 4.74	32.84 ± 6.32	1.000	0.036	0.296
Middle temporal	32.57 ± 5.46	31.77 ± 5.52	29.52 ± 6.93	1.000	0.341	0.738
Inferior temporal	27.90 ± 6.78	27.05 ± 7.05	24.47 ± 8.97	1.000	0.484	0.888
Parahippocampus	37.44 ± 5.58	34.20 ± 4.22	33.76 ± 7.32	0.241	0.151	1.000
Lingual Gyrus	39.96 ± 8.01	33.42 ± 5.83	31.73 ± 9.23	0.028	0.005	1.000
Fusiform	34.53 ± 5.88	31.13 ± 4.80	29.55 ± 7.48	0.242	0.039	1.000
Hippocampus	39.19 ± 6.62	32.69 ± 7.31	34.13 ± 9.72	0.034	0.147	1.000
Precuneus	38.84 ± 8.75	33.89 ± 5.90	33.89 ± 11.20	0.236	0.247	1.000
Supramarginal	35.79 ± 7.51	34.72 ± 5.64	32.13 ± 7.22	1.000	0.289	0.724

Table 6: Multiple comparison of ROIs for CBF analysis using bonferroni correction among HC, MCI and AD.

ROIS for VBM analysis	HC GM Volume (ml)	MCI GM Volume (ml)	AD GM Volume (ml)	Bonferroni corrected P value		
				MCI versus HC	AD versus HC	AD versus MCI
Superior frontal	26.59 ± 2.07	26.49 ± 3.47	24.85 ± 2.30	1.000	0.135	0.185
Middle frontal	19.91 ± 1.64	20.02 ± 2.74	18.40 ± 1.96	1.000	0.094	0.069
Posterior Cingulate	8.65 ± 0.77	8.58 ± 1.23	8.19 ± 1.19	1.000	0.547	0.798
Superior temporal	14.30 ± 1.21	13.67 ± 1.78	13.03 ± 1.59	0.582	0.035	0.605
Middle temporal	12.73 ± 1.11	12.68 ± 1.91	11.47 ± 1.45	1.000	0.034	0.047
Inferior temporal	11.29 ± 1.04	11.03 ± 1.61	10.05 ± 1.33	1.000	0.015	0.081

Parahippocampus	3.80 ± 0.31	3.63 ± 0.42	3.35 ± 0.42	0.450	0.001	0.087
Lingual Gyrus	7.29 ± 0.80	7.15 ± 0.76	6.65 ± 0.95	1.000	0.059	0.208
Fusiform	6.93 ± 0.63	6.73 ± 0.93	6.17 ± 1.04	1.000	0.024	0.151
Hippocampus	3.81 ± 0.32	3.53 ± 0.58	2.98 ± 0.66	0.299	<0.001	0.007
Precuneus	6.07 ± 0.71	6.18 ± 1.03	5.63 ± 0.96	1.000	0.415	0.204
Supramarginal	7.40 ± 0.64	7.51 ± 0.89	6.92 ± 0.94	1.000	0.225	0.092

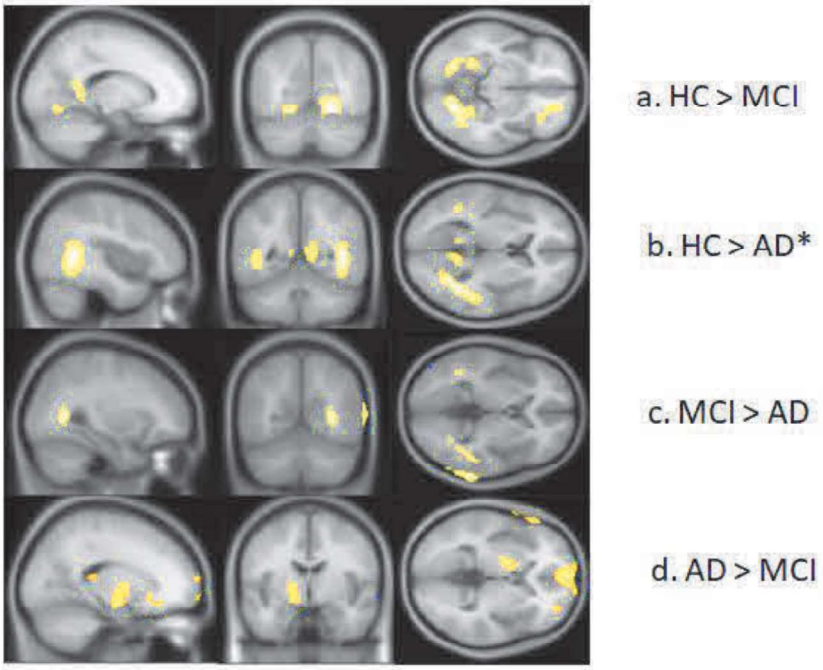

Figure 8: Analysis of variance (ANOVA) (three groups, covariates: TIV, age and gender): areas of hypo-perfusion and hyperperfusion in patients with MCI and AD as compared with cognitively healthy controls. * FDR corrected ($p < 0.05$)

Table 7: Analysis of variance (ANOVA) (three groups, covariates: TIV, age and sex): areas of CBF variations in patients with mild cognitive impairment (MCI) and mild dementia in Alzheimer's disease (AD) compared with cognitively healthy controls (HC) at P<0.001 uncorrected, cluster size threshold: 20

Anatomic Location	Voxel EquivZ	MNI coordinates		
		x	y	Z
HC>MCI				
R Superior frontal	2.86	-28	-46	-12
R Middle frontal	3.42	32	54	2
R Precuneus	2.75	6	-54	2

R Fusiform	3.74	22	-66	-12
R Lingual	2.6	-2	54	2
L Fusiform	3	-28	-46	-12
L Lingual	2.6	-14	-46	0
HC>AD				
R Superior temporal *	4.14	50	-32	-2
R Middle temporal *	3.86	52	-32	2
R Inferior temporal	3.82	44	-52	-10
R Supramarginal	3.95	46	-34	36
R Inferior parietal	3.92	40	-42	40
R Posterior cingulate *	3.99	4	-36	28
R Precuneus *	3.14	6	-52	14
R Fusiform *	3.7	22	-64	-14
R Lingual gyrus *	3.6	-14	-72	-8
R Parahippocampus	3.32	12	-48	2
L Middle temporal	3.44	-44	-50	4
L Posterior cingulate *	3.46	-6	-40	14
L Lingual *	3.17	-14	-72	-8
MCI > AD				
R Middle temporal	2.67	68	-40	8
AD > MCI				
L Hippocampus	2.37	-18	-8	-14
L Pallidum	2.23	-18	-4	0

*P < 0.05 False Discovery Rate (FDR) corrected

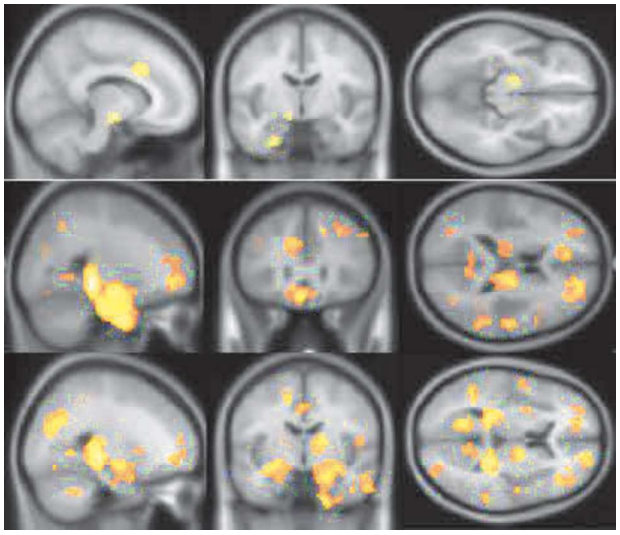

Figure 9: Analysis of variance (ANOVA) (three groups, covariates: TIV, age and gender): areas of gray matter atrophy in patients with MCI and AD as compared with cognitively healthy controls. * FDR corrected ($p < 0.05$)

Table 8: Analysis of variance (ANOVA) (three groups, covariates: TIV, age and sex): areas of gray matter atrophy in patients with mild cognitive impairment (MCI) and mild dementia in Alzheimer's disease (AD) compared with cognitively healthy controls (HC) at P<0.001 uncorrected, cluster size threshold: 20

Anatomic Location	Voxel EquivZ	MNI coordinates		
		x	y	Z
HC>MCI				
R Precuneus	3.2	6	-53	2
R Fusiform	3.5	22	-64	-12
L Fusiform	3.83	-24	-6	-37.5
HC>AD				
R Superior temporal *	2.85	57	-1.5	-6
R Middle temporal *	4.2	63	-28.5	-13.5
R Inferior temporal *	4.55	63	-25.5	-19.5
R Supramarginal *	3.17	64.5	-28.5	36
R Inferior parietal *	2.97	39	-46.5	52.5
R Posterior cingulate *	3.55	4.5	-48	30
R Precuneus *	3.12	12	-61.5	42
R Fusiform *	3.03	31.5	-33	-19.5
R Lingual gyrus *	2.57	16.5	-61.5	-6
R Parahippocampus	4.56	21	-34.5	-10.5
L Middle temporal *	3.85	-58.5	-25.5	-10.5
L Posterior cingulate *	3.14	-6	-46.5	30

L Lingual *	3.27	-16.5	-43.5	-6
MCI > AD				
R Middle temporal *	2.36	55.5	-24	-15

*P < 0.05 False Discovery Rate (FDR) corrected

V. ^{1}H MR spectroscopic findings

- The spectroscopic data were processed using functool in aw workstation and the major peaks of N-acetyl aspartate (NAA), Cr, Cho, and mI were identified to evaluate the possible alterations in the posterior cingulate. Then, the ratios of NAA/ Cr, Cho/Cr, mI/Cr, and NAA/mI were analyzed.

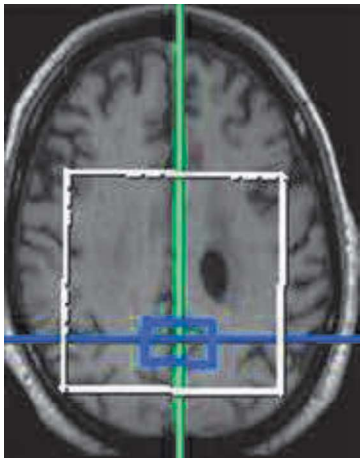

Figure 10: Posterior cingulate gyrus voxel placing of 1H-MRS findings in patients with Alzheimer's disease

Table 9: Metabolite ratios from the posterior cingulate voxel in the controls, mild cognitive impairment, and Alzheimer's disease

Metabolite Ratio	Controls	MCI	AD
NAA/Cr	2.56 ± 1.30	2.28 ± 0.29	2.14 ± 0.30
Cho/Cr	0.43 ± 0.12	0.45 ± 0.10	0.58 ± 0.26
mI/Cr	0.25 ± 0.09	0.31 ± 0.10	0.41 ± 0.39
NA/mI	10.85 ± 6.08	7.66 ± 1.83	7.68 ± 3.82

MCI=Mild cognitive impairment, AD=Alzheimer's Disease,NAA=N-acetyl asparate, Cr=Creatine, Cho=Choline, mI =Myoinositol

11. Conclusions summarizing the achievements and indication of scope for future work:

- ICA analysis revealed 11 different resting sate networks. Further, the ICA analysis showed a group difference among DMN and salience networks. ROI to ROI whole brain functional connectivity analysis in MCI and AD revealed significantly reduced functional connectivity

in interlobe connections such as from temporal to frontal and from temporoparietal to basal ganglia regions. Compared to MCI, AD group exhibited reduced connections from posterior cingulate to precuneus. Fundamental network parameters of networks: global efficiency, local efficiency, betweenness centrality, cost, clustering coefficient, and degree was lower in MCI and AD compared to controls.

- VBM analysis showed reduced gray matter (GM) density in the left superior temporal gyrus, left post central gyrus, left parahippocampus and in the left fusiform in MCI whereas, the AD subjects revealed significant GM reduction in hippocampus, para hippocampus, amygdala, heschl gyrus bilaterally, right calcarine, right supramarginal gyrus and right middle compared to controls.
- The DTI analysis revealed reduced Fractional Anisotropy and increased mean diffusivity in the patient group than in controls for cingulum, superior longitudinal fasciculus, inferior longitudinal fasciculus, forceps minor, forceps major and uncinated tracts.
- ASL analysis depicted hypoperfusion in the precuneus, posterior cingulate gyrus, inferior temporal, middle temporal parietal and middle occipital lobe in patients with MCI and AD. In voxel-wise comparison, patients with MCI showed both hypoperfusion and atrophy in precuneus, fusiform and lingual gyrus and AD group revealed atrophy in all the areas reported with hypoperfusion. After Bonferroni correction, robust hypoperfusion were found in posterior cingulate and lingual gyrus in all stages of AD (MCI and fully developed AD). Besides, we observed significant elevated perfusion in hippocampus in patients with AD compared to MCI.
- The MRS analysis revealed lower NAA/mI levels mI and higher Cho/Cr levels in the PCC in MCI and AD patients than in controls. Besides, a higher mI/Cr level and lower NA/mI level was depicted in patients with MCI and AD compared to controls.

12. S&T benefits accrued:

i. List of Research publications

S No	Authors	Title of paper	Name of the Journal	Volume	Pages	Year
1	Sheelakumari R, C. Kesavadas, Lekha V.S, Sunitha Justus, P.Sankara Sarma, Ramshekhar Menon.	Structural correlates of Mild Cognitive Impairment - A clinicovolumetric study	Neurology India	66	370-6	2018
2	Sheela Kumari R,	Multimodality	Annals of Indian	21	133-9	2018

	P.Sankara Sarma, C. Kesavadas, Bejoy Thomas, Deepak Sasi, Lekha V.S., Sunitha Justus, Mridula Mathew, Ramshekhar N Menon	Neuroimaging in Mild Cognitive Impairement- A cross sectional comparison study.	Academy of neurology			
3	S. Nanda, R.Menon, C.Kesavadas, S.Kumari	A pilot study on mapping structural and functional connectivity in early Alzheimer's disease(AD) in comparison to stable mild cognitive impairment (MCI) and healthy controls	Journal of the Neurological Sciences	381	766	2017

The results of the study were presented as part of the conferences:

- Sheela Kumari R, C.Kesavadas, Nandini, Ramshekhar Menon. While brain Resting-State Functional Connecivity Networks in Mild Cognitive Impairement and Alzheimer's Disease. Brainmodes 2017, National Brain Research Centre, Gurgaon, India
- Satyan Nanda, Sheela Kumari R, C.Kesavadas, , Ramshekhar Menon. A Pilot Study on Mapping Structural and Functional Connectivity in Early Alzheimer's Disease (AD) in comparison to stable Mild Cognitive Impairement (MCI) and healthy controls. World Congress of Neurology 2017, Kyoto, Japan. Dr Nanda was the recepient of the Young Investigator Bursary award to attend this prestigious Congress.
- R. Sheela Kumari R, A. Subramoniam, P.G. Rajesh, C. Kesavadas, R.N. Menon. Resting State Functional connectivity and Gray matter changes associated with Mild Cognitive Impairment and Alzheimer's Disease. "Cognition and Dementia"2nd T.S. Srinivasan- Knowledge Conclave 2016, NIMHANS, Banglore.
- Sheela Kumari R, C.Kesavadas, Nandini, Ramshekhar N. Menon. "Whole Brain Resting State Functional Connectivity Networks in Mild Cognitive Impairment and Alzheimer's Disease". Poster presented at "Brain Modes", 2017, 11-14 December, NBRC, Gurgaon.

Papers under review:

1. Shania M Soman, R. Sheela Kumari, C Kesavadas, Nandini, Ramshekhar N. Menon, “Patterns of cerebral hypo perfusion and gray matter atrophy in Mild cognitive impairment and Alzheimer's Disease”, J Alzheimers Dis, 2018.
2. Shania M Soman, R. Sheela Kumari, C Kesavadas, Nandini, Ramshekhar N. Menon, "An assessment of resting state networks in patients with MCI and AD using ICA analysis and seed connectivity”, Behav Brain Res, 2018.
3. Shania M Soman, R. Sheela Kumari, C Kesavadas, Nandini, Ramshekhar N. Menon, " Multi-modality MRI neuro-imaging biomarkers for the diagnosis of patients with Mild cognitive impairment and Alzheimer's disease", J Alzheimers Dis, 2018.

ii. Manpower trained on the project

a) Research Scientists or Research Associates
Senior Research Fellow – 1
Junior Research Fellow-1

b) No. of Ph.D. produced
Nil

c) Other Technical Personnel trained
Nil

iii. Patents taken, if any
Nil

13. Financial Position:

No	Financial Position/ Budget Head	Funds Sanctioned	Expenditure	% of Total cost
I	Salaries/ Manpower costs	12,09,200	12,09,200	100%
II	Equipment	4,50,000	4,50,000	100%
III	Supplies & Materials	3,00,000	3,00,000	100%
IV	Contingencies	40,000	40,000	100%
V	Travel	40,000	40,000	100%
VI	Overhead Expenses	1,50,000	1,50,000	100%
VII	Others, if any			
	Total	21,89,200	21,89,200	**100%**

14. Procurement/ Usage of Equipment a) Nil

S No	Name of Equipment	Make/Model	Cost (FE/ Rs)	Date of Installation	Utilisation Rate (%)	Remarks regarding maintenance/ breakdown

b) Plans for utilising the equipment facilities in future Nil

Name and Signature with Date

a. 18/12/18
(Principal Investigator)

b. 21/12/18
(Co-Investigator)

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Structural correlates of mild cognitive impairment: A clinicovolumetric study

R Sheelakumari, C Kesavadas[1], V S Lekha, Sunitha Justus, P Sankara Sarma[2], Ramshekhar Menon

Abstract:

Context: Annually 10–12% of patients with mild cognitive impairment (MCI) are likely to progress to Alzheimer's Disease (AD). The morphometric profile in stable non-converters has not been adequately characterized.

Aims: To determine the structural differences between amnestic MCI and early AD using volumetric magnetic resonance imaging (MRI) and its correlation with neuropsychological test performances.

Settings and Design: This was a hospital-based case-control study.

Materials and Methods: Twenty-four patients classified as having "non-progressor" MCI, 13 as having an early AD, and 25 controls, and assessed using neuropsychological evaluation, and three-dimensional T1-weighted 1.5T magnetic resonance maging (MRI) were included in the study. We used both voxel-based morphometry and automated regional volumetry to assess the topographical patterns of volume loss.

Statistical Analysis Used: Post-hoc analysis of variance was done for comparison between means, and partial correlation analysis was done for correlating volumetric and cognitive measures.

Results: Consistently, significant atrophy of the superior temporal gyrus, left hippocampus, and mesial frontoparietal regions were identified in patients with MCI in comparison to controls. Increased atrophy in the limbic regions, temporal neocortex, and precuneus was identified in patients with early AD in comparison to patients with MCI. While differences in retention and recall scores between the groups were independent of age and volumetric variables, significant correlations were observed between the learning and recall scores and the volume of hippocampus in patients with MCI as well as temporal neocortex in patients with AD. Atrophy of the superior temporal gyrus and mesial neocortical regions represents the structural correlate of amnestic MCI parallel to the development of hippocampal atrophy.

Conclusions: Identification of the pattern of volumetric abnormalities in patients with amnestic MCI in addition to atrophy of the medial temporal lobes necessitates a close follow up to continuously assess these patients for their progression to early AD.

Key Words:

Alzheimer's disease, amnestic mild cognitive impairment, automated regional volumetry, voxel-based morphometry

Key Messages:

This is the first clinicovolumetric study on mild cognitive impairment from India. Realistic differences exist between apparently stable mild cognitive impairment, early Alzheimer's disease, and cognitively normal healthy controls, irrespective of the methodology used in voxel-based morphometry. Learning and recall measures notably correlate with the hippocampal volume in patients with mild cognitive impairment, as well as with the temporal neocortex volume in patients with AD.

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Mild cognitive impairment (MCI) is considered to be a transitional stage between normal aging and Alzheimer's Disease (AD),[1] and its rate of progression varies between individuals. The longitudinal risk of developing AD in amnestic MCI (a-MCI) can be estimated by measures of memory performance and subjective level of cognitive functioning.[2-4] Neuropsychology tests such as vernacular adaptations of Addenbrooke's Cognitive Examination (ACE) and Rey Auditory Verbal Learning Test (RAVLT)[5,6] have been found to be effective screening and definitive measures of cognitive impairment in a-MCI. Learning and retention measures, as assessed in RAVLT, have been shown to be among the most comprehensive clinical predictors of conversion from a-MCI to AD.[6] While it is believed that

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roughly 12% of patients with MCI annually run the risk of conversion to AD, with approximately 80% converting to dementia by 6 years, other studies debate this conversion rate.[7] It is also well-known that a large subset of patients remains stable over the years or may clinically revert to normal cognitive status.[8]

The most well-known approaches employed in the analysis of gray matter (GM) loss in MCI and AD is voxel-based morphometry (VBM) and automated regional volumetry (ARV).[9] Not surprisingly, prominent studies till date have focused on MTL and amygdala volumes.[10] Little is known regarding the relationship between GM volume in other cortical regions and performance on the neuropsychological tests of patients with MCI or AD. Therefore, the aim of the present cohort study was to quantify the structural changes in cognitively stable a-MCI and AD patients compared to individuals with no cognitive impairment (NCI) in South India, employing quantitative volumetric magnetic resonance imaging (MRI) analysis tools such as ARV and VBM. The study also aimed to correlate the neuropsychological test performances using screening tests and definitive tools for memory impairment with morphometric variables in patients with MCI in comparison with a diseased group of patients suffering from an early AD.

Patients and Methods

Case selection

Sixty-two participants (NCI: 25, MCI: 24, AD: 13) were prospectively recruited from Memory and Behavioral Neurology clinic at a reputed centre in the city of Trivandrum, located in the southern state of Kerala, India. Early AD patients were selected using the standard NINCDS–ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) diagnostic criteria with a clinical dementia rating (CDR) score of <2.[11] Patients with a-MCI, aged between 55–80 years, diagnosed as per the modified Petersen's criteria[1] with a CDR of ≤0.5, who were still functioning independently in the community, and having a normal general cognition (i.e., MMSE >24) with normal performance on tests of other cognitive domains, viz., language, executive functions, visuospatial functions, praxis, and perception were included. The minimum duration of follow up of MCI patients required for inclusion into the study was 1.5 years. It was ensured prior to their inclusion that none of them had neuropsychological or CDR evidence of progression to dementia. The objective evidence of memory impairment required that an individual scored less than mean –1.5 standard deviation (SD) from the norm on at least two tests in the memory domain. This method has been validated previously.[6] The inclusion criteria for the cognitively normal healthy controls included an age range of 55–80 years, with formal education of more than 8 years, with no history of subjective memory complaints, and no major neurological, psychiatric, or medical comorbidities. All participants underwent a detailed, clinical, neuropsychological, and radiological evaluation after providing a written informed consent. The study was approved by the Institutional Ethics Committee.

Cognitive assessments

The participants underwent cognitive screening by the vernacular adaptation of ACE, which has been validated previously.[5] This battery has a measure of global cognition (mini-mental state examination, MMSE), and also includes tests for memory (immediate and delayed recall of a seven-item address list), verbal fluency (initial letter *P* and categories of animals), confrontation naming (ten items), and constructional praxis (copying two line-drawings). It also assesses executive functions and constructional ability (clock-drawing), remote memory, and language. Registration/learning is scored on a 24-point scale which has 3 points for registration of 3 words and 21 points for 3-trial learning of an address. The recall score was drawn from a 10-point scoring which included a 5-min recall of the three items presented previously and a 7-point recall of the address. Participants were required to have a depression score on the Hospital Anxiety Depression Scale (HADS) of less than 7. The measures of learning and recall studied in this cohort were the cumulative learning trials and delayed recall scores in the vernacular adaptation of Rey Auditory Verbal Learning Test (RAVLT) and memory subsets of ACE. Multidomain involvement in MCI was excluded as diagnosis of a-MCI necessitated normal performance on the language, executive, and visuospatial domains. A semantic battery employing confrontation naming for language; trail making A and B, and Wisconsin card sorting test (WCST) for executive functions; and, visual objective space perception battery, and judgement of line orientation for visuospatial functions were the tests used to exclude the presence of patients with either a multidomain involvement, or having the presence of non-a-MCI.

Image acquisition

Participants were scanned with a 1.5T (Seimens Magnetom-Avanto SQ engine, Erlangen, Germany) MRI scanner in the Department of Radiology. The three-dimensional (3D) T1 weighted anatomical scans were acquired using a flash-spoiled gradient echo sequence with 1mm slice thickness, 176 sagittal slices, 1 × 1 × 1mm voxel size, 11 ms repetition time, 4.95 ms echotime, 15° flip angle, matrix size of 256 × 256 with a total scan time of 6 min 21 s.

Image processing

Whole brain analysis

VBM8 toolbox in Statistical Parametric Mapping (SPM8- Wellcome Department of Imaging Neuroscience, London) was applied to investigate the cortical GM changes across the entire brain without any prior knowledge of the region of interest (ROI). All the images were oriented in the anterior commissure-posterior commissure (AC-PC) line. The images were segmented into GM, white matter (WM), and cerebrospinal fluid (CSF), and spatially normalized into the Montreal Neurological Institute (MNI) space using VBM8 DARTEL procedure with custom settings. The segmentation was followed by modulation for preserving the volume of a particular tissue in a fixed voxel, and volumes of GM, WM, CSF, and total intracranial volume (TIV) were calculated. The resulting images were smoothed with an isotropic Gaussian kernel of 8mm full width half maximum (FWHM).

Group comparison among MCI, AD, and controls were performed by one-way analysis of variance (ANOVA) within the Statistical Parametric Mapping 8 (SPM8) general linear model. Group comparisons were tested with a corrected threshold of $P < 0.001$, which determines the clusters with significant differences in GM concentration. Three separate

contrasts (NCI vs MCI, NCI vs AD, and MCI vs AD) with age, sex, and total intracranial volume (TIV) as covariates were used to compare the GM density between participants. The significant atrophic regions were overlaid on T1-weighted standard brain images, allowing the localization of areas of significant GM loss. The atrophic regions are reported in Montreal Neurological Institute (MNI) coordinates with the help of xjview toolbox (http://www.alivelearn.net/xjview/).

Volumes of cortical gray matter structures using automated regional volumetry

The segmentation algorithm in SPM8 and Automated Anatomic Labelling (AAL),[12] (http://www.cyceron.fr/web/aal) template was used to estimate the bilateral volumes of eight anterior and medial temporal structures, namely, inferior temporal gyrus, superior temporal gyrus, entorhinal cortex, middle temporal pole, superior temporal pole, parahippocampus, hippocampus, and amygdala, one deep gray matter structure (thalamus), and two parietal structures (precuneus and posterior cingulate). After segmentation, the volume of each region was obtained by writing scripts in MATLAB window.

Statistical analysis

Demographic data, neuropsychological measures, and cortical GM volumes between study groups were analyzed using the Statistical Package for the Social Sciences Statistics for Windows version 21 (SPSS, IBM. Armonk, NY, USA). The demographic and cognitive variables were compared across the groups, NCI, MCI, and AD, using univariate analysis of variance (ANOVA). Bonferroni post-hoc procedure with age correction was applied to compare means of the independent variables of volume with diagnosis. Partial correlation was used to assess the relationship between independent volumes and neuropsychological test measures in each study group. In all comparisons, the level of statistical significance was set at $P < 0.05$.

Results

Demographics, cognitive function, and memory

Demographic and neuropsychometric measures between the three groups are depicted in Table 1. The mean duration of subjective memory complaints in the MCI group was 5.8 ± 3.7 years and the mean disease duration in the AD group was 4.2 ± 1.5 years. The mean duration of follow-up since the time of diagnosis to the time of inclusion into the study was 3.5 ± 1.9 years in the MCI group, and 2.2 ± 0.7 years in the AD group. After adjusting for age, patients with both MCI and AD were significantly impaired on ACE recall, ACE total, RAVLT cumulative learning, and delayed recall compared to individuals with NCI ($P < 0.001$). Similarly, the AD group performed poorly in comparison to the MCI group on all components of cognitive and memory scores [ACE recall, ACE total, RAVLT recall ($P < 0.001$) and RAVLT learning scores ($P = 0.013$)]. None of the MCI patients had progressed to early AD during the minimum 1.5-year period of follow-up at the time of inclusion into the study.

Whole brain findings

The participant groups were contrasted to reveal the patterns of GM atrophy on VBM group analysis. Compared to controls, MCI patients revealed predominant atrophy in bilateral superior temporal gyri. Significant atrophic patterns were also observed in the hippocampus, inferior temporal gyrus, middle frontal gyrus, cuneus and lingual gyrus in the left hemisphere, the middle occipital gyrus and cingulate gyrus in the right hemisphere [Figure 1 and Supplementary Table 1]. For AD patients, most significant GM loss was detected in the left parahippocampal gyrus. In addition, AD patients had

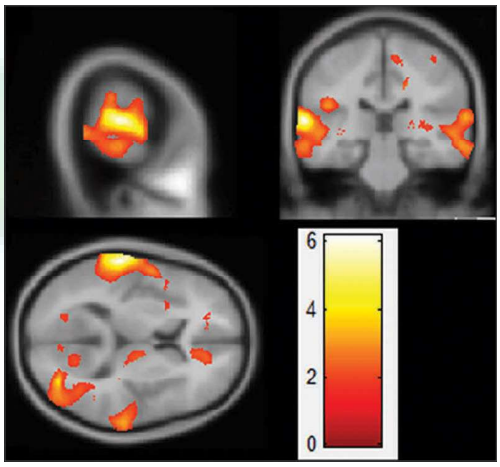

Figure 1: VBM comparison between patients with MCI and normal healthy volunteers. Projection of SPM T-map on the customized MRI template showing the atrophic patterns with an uncorrected threshold of $P < 0.001$

Table 1: Demographics and Cognitive test scores each in the groups [mean±SD and pairwise post-hoc Bonferroni results]. Test scores represent raw scores

	NCI	MCI	AD	Bonferroni corrected *P*		
				MCI vs. NCI	AD vs. NCI	AD vs. MCI
N	25	24	13	-	-	-
Sex (M/F)	13/9	8/7	7/4			
Mean age (mean±SD in years)	63.24±6.94	69.83±5.76	69.23±5.60			
Education (years)	12.80±3.68	11.29±3.25	12.85±3.64			
ACE registration	20.12±3.84	18.42±3.17	16.46±3.33	0.14	0.003	0.17
ACE recall	7.48±2.23	3.92±1.99	1.08±1.65	<0.001	<0.001	0.004
Total ACE score	92.52±7.18	82.54±5.83	69.38±9.48	0.001	<0.001	<0.001
RAVLT cumulative learning score	49.92±6.22	36.88±10.04	27.38±8.39	<0.001	<0.001	0.013
RAVLT 20 min recall score	11.76±2.90	5.88±2.98	2.15±2.03	<0.001	<0.001	<0.001

NCI = No cognitive impairment, MCI = Mild cognitive impairement, AD = Alzheimer's Disease, ACE = Adenbrook's Cognitive Examination, RAVLT = Rey Auditory Verbal Learning Test, N = Number, SD = Standard deviation, M = male, F = Female, vs: Versus

significant GM loss in bilateral hippocampi, bilateral precunei, bilateral fusiform gyri, bilateral middle temporal gyri, bilateral anterior cingulate regions, right inferior temporal gyri, right superior temporal pole, right posterior cingulate, right rolandic operculum, right thalamus, right inferior parietal lobule, right middle occipital gyri, left middle frontal, and orbitofrontal gyri [Figure 2 and Supplementary Table 2]. Moreover, a direct comparison of the patient groups revealed greater cerebral atrophy in AD patients [Figure 3 and Supplementary Table 3] in bilateral parahippocampi, hippocampi, amygdalae, fusiform gyri, superior and middle temporal poles, inferior temporal gyri, and medial orbitofrontal regions along with atrophy in the right precuneus and left angular regions.

Gray matter volumes using automated regional volumetry

The regional volumes of GM structures estimated by using ARV are summarized in Table 2. Post-hoc Bonferroni procedure showed significant volume differences for the left hippocampus, right precuneus, and bilateral superior temporal gyri in the MCI patients compared to individuals with NCI. In patients with AD, bilateral hippocampi, precunei, superior temporal gyri, parahippocampi, right amygdala, and right superior temporal pole revealed significant GM volume loss compared to controls. Only the total GM and left hippocampal volume discriminated the patient groups, with lower volumes detectable in AD patients.

Correlation between neuropsychology parameters and automated lobar volumetry

Partial correlation analysis with age as the controlling variable showed that RAVLT delayed recall positively correlated with reduced volumes of left ($r_p = 0.42$, $P < 0.04$) and right ($r_p = 0.38$, $P < 0.05$) hippocampi in MCI patients. A similar observation was noted between RAVLT total learning and the hippocampal volume ($r_p = 0.42$, $P < 0.04$) in the left hemisphere; whereas, in AD, a significant correlation was observed between RAVLT total learning and the right hemisphere volume of the middle temporal pole ($r_p = 0.66$, $P < 0.01$) and superior temporal pole ($r_p = 0.84$, $P < 0.001$). In addition, ACE total score significantly correlated with volume of the right superior temporal pole ($r_p = 0.73$, $P < 0.006$).

Discussion

Our study was designed to quantify the GM differences in stable "non-progressor" a-MCI patients compared to early AD patients and NCI individuals using VBM group analysis and ARV. Further, the study compared the cognitive measures that were deemed clinically relevant for tracking the progression of MCI to AD. Previous studies have demonstrated variable results using these two methods, predominantly favouring VBM over ARV for assessing GM volume differences between the groups.[13,14] The features unique to a-MCI patients who are clinically stable are crucial to determining structural measures of stability in what is usually considered to be a pre-AD status. To the best of our knowledge, this is the first study in the Indian population that utilized automated software tools to discriminate MCI and AD population from individuals with NCI, as well as to correlate the GM loss in specific anatomical regions with cognitive measures. A recent Indian study addressed the relationship between regional morphometric brain changes and cognitive deficits in AD patients in relation to NCI.[15]

As VBM analysis is a practical tool for whole-brain group comparisons, identifying regions which can be subsequently analyzed using ARV, we identified certain regions of interest (ROIs) in each comparative model. Considering the MCI patients, GM volume loss was detected in the superior temporal gyrus and left hippocampus, and variably over the mesial brain regions (cuneus, precuneus, and cingulate cortex), in comparison to individuals with NCI. The observation of hippocampal atrophy is in line with previous reports[16-18] and is established as a marker of future progression in MCI. Atrophy identified in the mesial cortical regions, especially the precuneus, as identified on ARV, is a unique finding in this study and represents an avenue for future research considering the role of precuneus in episodic memory retrieval and visuospatial awareness, and from a network perspective, in the default mode network.[19] This highlights the added functional significance of the posteromedial regions of the cortex in MCI in addition to atrophy of the temporal lobe. The inferior frontal gyrus atrophy identified in the MCI group is in line with the

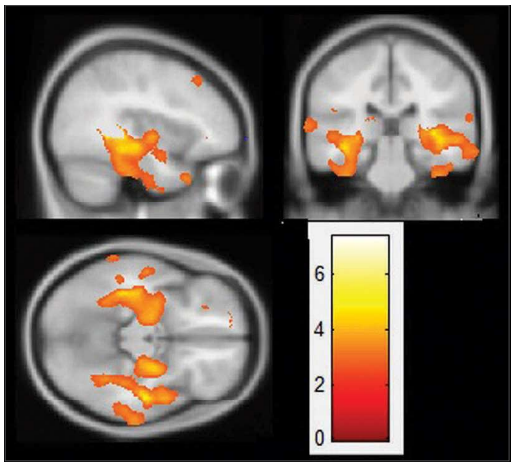

Figure 2: VBM comparison between patients with AD and normal healthy volunteers. Projection of SPM T-map on the customized MRI template showing the atrophic patterns with an uncorrected threshold of $P < 0.001$

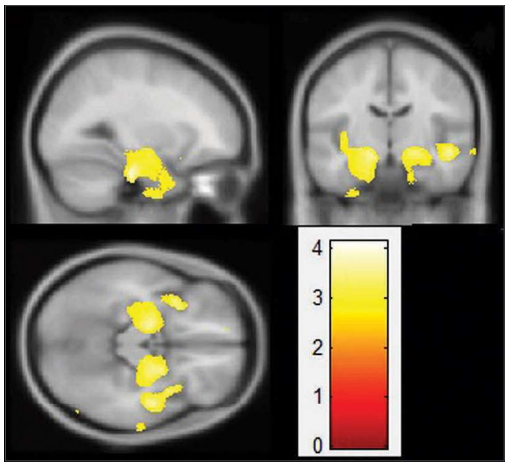

Figure 3: VBM comparison between patients with AD and MCI patients. Projection of SPM T-map on the customized MRI template showing the atrophic patterns with an uncorrected threshold of $P < 0.001$

Table 2: Anatomical structures with significant group differences of volume in MCI and AD patients compared with normal healthy non-demented controls. Each group shows mean (SD) values in cu.mm, and pairwise posthoc Bonferroni correction results with age as covariate

Dependent variable	NCI	MCI	AD	*MCI vs. NCI	*AD vs. NCI	*AD vs. MCI
GM	605.23±53.69	553.63±39.94	511.92±51.95	**<0.001**	**<0.001**	**0.003**
WM	470.42±64.66	462.05±74.69	430.98±76.24	1.00	0.331	0.629
CSF	529.80±107.71	579.51±96.49	584.47±131.82	0.411	0.511	1.00
TIV	1607.69±160.32	1595.21±127.92	1522.81±177.75	1.00	0.35	0.542
Hippocampus						
L	3.86±0.348	3.53±0.491	3.12±0.483	**0.043**	**<0.001**	**0.03**
R	3.75±0.33	3.55±0.59	3.27±0.625	0.519	**0.028**	0.34
Parahippocampus						
L	4.11±0.57	3.91±0.514	3.51±0.515	0.58	**0.02**	0.86
R	4.81±0.60	4.52±0.50	4.16±0.60	0.182	**0.003**	0.14
Amygdala						
L	1.11±0.162	1.09±0.126	1.04±0.154	1.00	0.591	1.00
R	1.23±0.174	1.12±0.122	1.06±0.17	0.073	**0.014**	0.85
Thalamus						
L	2.54±0.321	2.37±0.432	2.31±0.30	0.227	0.149	1.00
R	2.87±0.531	2.56±0.411	2.52±0.30	0.199	0.151	1.00
Precuneus						
L	11.43±1.20	10.51±1.69	10.33±1.34	0.165	**0.026**	1.00
R	10.70±1.08	9.72±1.34	9.39±1.19	**0.044**	**0.002**	1.00
Entorhinal cortex						
L	1.20±0.20	1.14±0.14	1.08±0.13	0.607	0.103	0.85
R	1.22±0.21	1.15±0.13	1.10±0.13	0.42	0.142	1.00
Posterior cingulate						
L	1.32±0.35	1.27±0.18	1.26±0.17	1.00	1.00	1.00
R	0.84±0.09	0.80±0.17	0.75±0.11	0.539	**0.01**	0.60
Inferior temporal gyrus						
L	10.92±1.62	10.59±1.27	9.83±1.29	1.00	0.086	0.37
R	12.64±1.92	12.27±1.23	11.40±1.40	1.00	0.135	0.07
Superior temporal gyrus						
L	11.24±1.04	10.21±1.26	9.76±0.91	**0.006**	**0.001**	0.73
R	9.25±1.01	8.34±0.63	8.15±1.27	**0.025**	**0.034**	1.00
Middle temporal pole						
L	2.47±0.61	2.43±0.40	2.30±0.39	1.00	0.942	1.00
R	3.29±0.81	3.15±0.53	2.77±0.41	1.00	0.062	0.26
Superior temporal pole						
L	4.39±0.97	4.28±0.57	3.93±0.58	1.00	0.251	0.55
R	3.88±0.88	3.68±0.52	3.17±0.50	0.92	**0.02**	0.11

NCI = No cognitive impairment, MCI = Mild cognitive impairement, AD = Alzheimer's Disease, GM = Grey Matter, WM = White Matter, CSF = Cerebrospinal Fluid, TIV = Total Intracranial Volume, L = left, R = Right. *Bonnferroni correction, $P<0.05$. The significant values are presented in bold figures

findings of Whitwell *et al*.,[20] as an indicator of faster disease progression. Only a longitudinal follow-up would indicate the significance of these results in our stable MCI group.

In the AD group, as in other studies, a significant GM atrophy was noted by both the techniques in the medial temporal (MTL) structures, i.e., the hippocampus, amygdala, parahippocampal gyrus,[21-23] and precuneus.[24] Similar to other studies, VBM identified atrophy of the anterior cingulate,[25,26] the fusiform gyrus, and the frontal[25,27] regions in AD compared to controls. Interestingly, in contrast to the literature,[28,29] our study failed to reveal significant impairment of the posterior cingulate region on group analysis. Furthermore, our study detected greater atrophy of the hippocampus in patients with AD compared to those with MCI, in addition to the medial and neocortical temporal lobe, anterior cingulate region, insula, and precuneus identified on VBM comparison between patients with AD and MCI. As has been shown in another study,[7] atrophy in these regions, if observed in MCI, may be indicative of transition to early AD.

As expected, patients with MCI and AD significantly differed from individuals with NCI on cognitive measures. The observations were retained even after controlling for age and regional volumes. The direct comparison between patient groups revealed significant differences in ACE recall, ACE total, and RAVLT total learning. These results would imply the existence of genuine clinical and functional differences between the 3 groups, and the independent relevance of these neuropsychological measures as predictors of cognitive decline

in the elderly.[30] Correlation between the cognitive scores and regional volume loss was very specific in the MCI and AD participants. Both RAVLT total learning and recall after 20 minutes significantly correlated with hippocampal atrophy in MCI participants; whereas, in AD, the decline in cognitive measures such as RAVLT total and ACE total significantly correlated with the volume of the superior temporal gyrus. These results indicate an association between cognitive functioning and regional morphometric brain changes, a probable epiphenomenon of pathological changes in MCI and AD considering the early deposition of amyloid plaques in neocortical areas preceding the limbic areas.[31,32]

Even though our study came up with the above-mentioned promising findings, it had some limitations. In the technique of ARV, we only selected certain regions in the temporal, parietal, and limbic regions; however, we excluded the frontal and occipital cortex. Although we included only stable MCI patients who were non-progressors during the duration of follow-up, a follow-up of greater than 5 years would have added further strength to the current study. However, the findings represent an avenue to predict conversion or stability in patients with atrophic MCI using population-based studies and to earmark patients at risk of progression.

Conclusion

In summary, the present study indicates that genuine neuropsychological differences exist between patients with MCI and AD, and control subjects. These differences are independent of age as well as the total GM volume and the regional cortical volumes, thereby highlighting functional differences that occur independent of structural differences between the groups. The volumetric findings of the study highlight the characteristics of stable MCI patients, with demonstration of atrophy in the temporal neocortex, hippocampus, and mesial neocortical regions as a key finding. In addition, atrophy in these regions along with frontoinsular regions could predict MCI due to AD. As we observed more findings in VBM, it is a more useful method for group comparisons. However, future cross-sectional and longitudinal studies in larger samples with comprehensive neuropsychological evaluation are needed to confirm these findings.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Multimodality Neuroimaging in Mild Cognitive Impairment: A Cross-sectional Comparison Study

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Abstract

Background and Purpose: Mild cognitive impairment (MCI) is a focus of considerable research. The present study aimed to test the utility of a logistic regression-derived classifier, combining specific quantitative multimodal magnetic resonance imaging (MRI) data for the early objective phenotyping of MCI in the clinic, over structural MRI data. **Methods**: Thirty-three participants with cognitively stable amnestic MCI; 15 MCI converters to early Alzheimer's disease (AD; diseased controls) and 20 healthy controls underwent high-resolution T1-weighted volumetric MRI, diffusion tensor imaging (DTI), and proton magnetic resonance spectroscopy (^{1}H MR spectroscopy). The regional volumes were obtained from T1-weighted MRI. The fractional anisotropy and mean diffusivity maps were derived from DTI over multiple white matter regions. The ^{1}H MRS voxels were placed over posterior cingulate gyri, and N-acetyl aspartate (NAA)/creatine (Cr), choline (Cho)/Cr, myoinositol (mI/Cr), and NAA/mI ratios were obtained. A multimodal classifier comprising MR volumetry, DTI, and MRS was prepared. A cutoff point was arrived based on receiver operator characteristics analysis. Results were considered significant, if $P < 0.05$. **Results:** The most sensitive individual marker to discriminate MCI from controls was DTI (90.9%), with a specificity of 50%. For classifying MCI from AD, the best individual modality was DTI (72.7%), with a high specificity of 87.9%. The multimodal classifier approach for MCI control classification achieved an area under curve (AUC) (AUC = 0.89; $P < 0.001$), with 93.9% sensitivity and 70% specificity. The combined classifier for MCI-AD achieved a highest AUC (AUC = 0.93; $P < 0.001$), with 93% sensitivity and 85.6% specificity. **Conclusions:** The combined method of gray matter atrophy, white matter tract changes, and metabolite variation achieved a better performance at classifying MCI compared to the application of individual MRI biomarkers.

Keywords: Magnetic resonance imaging, mild cognitive impairment, multimodality, spectroscopy, tensor imaging, volumetry

Introduction

Alzheimer's disease (AD) is the most common form of dementia affecting elderly people and presents with decline in memory and cognition.[1] Hence, it is important to identify patients at high risk of AD in the prodromal stage known as mild cognitive impairment (MCI), which is considered as a continuum between normal aging and dementia. However, the annual conversion rate of MCI to AD has been found to be approximately 10%–15%, with longitudinal trends in cognitive performances fluctuating between relative stability over time to decline.[2,3] Stable amnestic MCI has certain structural volumetric signatures.[4]

Several modalities of biomarkers have been proven to be sensitive for diagnosis of AD and MCI due to AD including brain atrophy measured by magnetic resonance imaging (MRI),[4,5] hypometabolism measured by positron emission tomography (PET),[6] and quantification of specific proteins measured through cerebrospinal fluid (CSF).[7,8] However, the nonavailability of the ligand C11-Pittsburgh compound B (PiB) and CSF amyloid beta (Aβ) or tau analysis in many centers across the world renders importance to other novel imaging biomarkers. In this study, we have used a combination of structural MRI analysis methods

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using voxel-based morphometry (VBM), diffusion tensor imaging (DTI), and proton magnetic resonance spectroscopy (^{1}H MRS) to measure volumetric changes, diffusion anisotropy of water molecules, and ratio of metabolites, respectively, in cognitively stable amnestic MCI compared to converters to early AD and healthy controls. We hypothesize that multiple MRI markers of underlying neuronal dysfunction can help improve the ability to identify patients with MCI as a distinctive entity, rather than using a single MRI marker. Furthermore, in the absence of expensive established biomarkers for AD such as PiB-PET, CSF Amyloid beta (Aβ)-42:Aβ-40 and CSF tau analysis that are not available at most centers in the developing countries, the MRI markers could be useful in diagnosis.. We also evaluate the relative sensitivity and specificity of multimodal MRI markers to enable objective phenotyping in MCI diagnosed using a standard array of neuropsychological tests.

Methods

Subjects

The study was designed as a cross-sectional observational study from a prospectively maintained database at a Memory and Neurobehavioral Disorders Clinic of a tertiary care hospital situated in the South Indian state of Kerala. The study had the approval from the Institutional Ethics Committee of our institute. Sixty-eight participants were recruited into the current study. These included 20controls; 33 amnestic MCI and 15 AD patients. Healthy controls were required to have a Mini–Mental State Examination (MMSE) score between 28 and 30, a clinical dementia rating (CDR) score of 0 with formal education of >8 years and no history of subjective memory complaints, and with no major neurological/psychiatric disorders were included in the study. The early AD patients were selected from a cohort of converters from amnestic MCI according to standard NINCDS–ADRDA diagnostic criteria with CDR of <2, to serve as diseased controls.[9] The MCI patients were diagnosed according to modified Petersen's criteria[2] with CDR of ≤0.5 and MMSE score between 24 and 29. Longitudinal cognitive stability without progression to overt dementia was required for a minimum period of 2 years before inclusion into the study for the MCI patients. The participants underwent cognitive screening by the vernacular adaptation of Addenbrooke's Cognitive Examination battery (ACE) and other domain-specific neuropsychological tests as detailed previously.[10,11]

Magnetic resonance imaging data acquisition

Only participants who had undergone structural MRI, DTI, and spectroscopic data were included in the study. All MRI scans were acquired on a 1.5T Siemens Magnetom Avanto scanner. Structural MR images were acquired using a FLASH sequence with TR/TE = 11/4.95 ms, slice thickness = 1 mm, flip angle = 15°, matrix size = 256 × 256, and voxel size = 1 mm × 1 mm × 1 mm. For DTI, we used a single-shot spin-echo echo-planar sequence with diffusion gradients along 30 noncollinear directions with parameters TR/TE = 6000/88 ms, slice thickness = 3 mm with 1.5 mm gap averaged twice a b value of 0 and 1000 s/mm^2. We also performed ^{1}H MRS acquisitions using PRESS sequence with water suppression by means of CHESS sequence. The two-dimensional chemical shift (2D CSI) multivoxel sequence with a TR/TE = 1590/30 ms, NEX = 3, bandwidth = 10 kHz, and data points = 2048 was used for the examinations. ^{1}H MRS voxels of 1.6 cm × 1.6 cm × 2.5 cm were placed over the posterior cingulate gyri on the midsagittal slice covering posterior cingulate gyri and inferior precunei bilaterally [Supplementary Figure 1].

Image analysis

The volumetric structural data were processed using voxel-based morphometry (VBM 8) toolbox in SPM8 (statistical parametric mapping software, Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm). First, the images were registered into the MNI space using high-dimensional DARTEL normalization algorithm. Then, the images were segmented into three different tissues: gray matter (GM), white matter (WM), and CSF [Figure 1]. After segmentation, the GM images were smoothed with a Gaussian kernel of 8 mm full width half maximum. The smoothed images were then multiplied with the binary masks of 11 region of interests (ROIs) bilaterally (including temporal

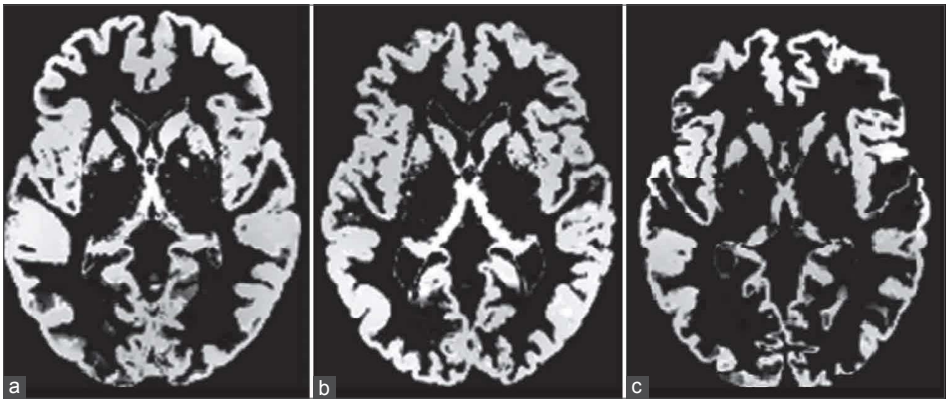

Figure 1: Segmented gray matter density maps in (a) controls (b) mild cognitive impairment and (c) Alzheimer's disease

neocortex, precuneus, and posterior cingulate) chosen priori from automated anatomic labeling atlas.[12] We computed the volume of each of the ROI region using MATLAB scripting. Postprocessing of DTI data was performed using a dedicated software package implemented in Siemens Leonardo workstation (Neuro 3D card). The system used an automatic correction of imaging distortions by scaling, de-skewing, and translational alignment of each of the image with the reference image (b = 0) to minimize the mismatch between the diffusion images and reference image. The offline tensor images and color maps were generated using the inbuilt "DTI task card." The anatomical images were overlaid on the DTI data and measured the fractional anisotropy (FA) and mean diffusivity (MD) at different locations using ROI-based analysis. The ROIs were placed on the specific anatomic locations by an experienced neuroradiologist (C. K) who was blinded to the clinical diagnosis. All data were analyzed by placing seven circular ROIs bilaterally with 5 mm pixels in temporal WM adjacent to temporal horn (TWM), genu and splenium of corpus callosum (CC), anterior and posterior subcortical WM (ASC and PSC), and anterior and posterior periventricular WM (APV and PPV) [Supplementary Figure 2]. The spectroscopic data were processed in the Leonardo workstation (Neuro3Dsoftware, Siemens) and the major peaks of N-acetyl aspartate (NAA), Creatine (Cr), Choline (Cho), and myoinositol (mI) were identified to evaluate the possible alterations in the posterior cingulate. Then, the ratios of NAA/Cr, Cho/Cr, mI/Cr, and NAA/mI were analyzed.

Statistical analysis

The demographic, neuropsychological, and radiological measures were compared across the three groups (MCI, AD, and NC) using univariate analysis of variance with *post hoc* Bonferroni correction. As there was significant age differences, age-adjusted comparisons were performed using general linear model. ROC curve analysis was carried out to classify MCI from controls and AD from MCI. For the ROC analysis, we used the mean GM density of the cortical regions, mean FA of the periventricular region, and mean metabolite ratios of NAA/mI and mI/Cr that differed in comparisons of MCI and controls and MCI and AD.

Furthermore, we computed a logistic regression that generated a probabilistic likelihood of MCI or AD diagnosis in each patient, and a score was derived as a linear combination of the regression coefficients and the imaging variables (T1-weighted MRI, DTI, and MRS). The obtained score was used to discriminate MCI from controls or AD. A cutoff point was arrived based on an ROC analysis. Results were considered significant, if $P < 0.05$.

Results

Demographic and neuropsychological results

Table 1 depicts demographics and neuropsychological test scores in patients and controls. The neuropsychological assessment results are listed in Table 1. Patients with MCI and AD were significantly impaired on ACE total, RAVLT total, and RAVLT recall after 20 min compared to controls. As neuropsychology test scores formed the objective basis for classification, the table is reflective of classification accuracy into each subgroup.

Gray matter density results

Patients with MCI had significantly reduced GM volume relative to controls in the right thalamus ($P = 0.02$) and posterior cingulate cortex (PCC; $P = 0.03$) [Supplementary Table 1]. Patients with AD compared to controls demonstrated significant volumetric differences in bilateral hippocampus ($P < 0.001$ for right and $P = 0.03$ for left), parahippocampus ($P = 0.03$ for right and $P = 0.04$ for left), and superior temporal gyrus ($P = 0.01$ for right and $P = 0.001$ for left), along

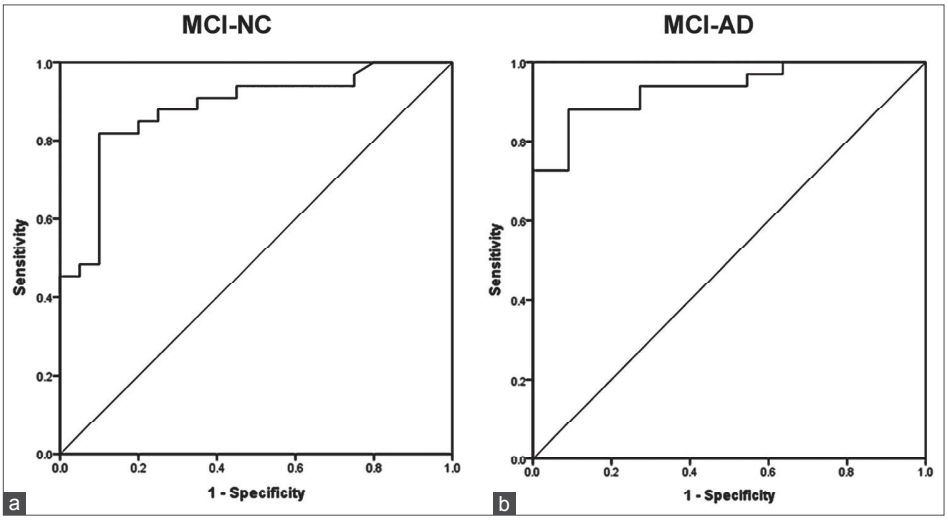

Figure 2: Receiver operator characteristic curves for classification of (a) mild cognitive impairment and controls and (b) mild cognitive impairment and Alzheimer's disease based on the optimal measure from each domain alone, and multimodal combination of gray matter density, fractional anisotropy, and metabolite ratios

with right amygdala (P = 0.002), thalamus (P = 0.02), and PCC (P = 0.006). A direct comparison between AD and MCI also revealed volume loss in hippocampus (P = 0.001 for right and P = 0.045 for left), inferior temporal gyrus (P = 0.004 for right and P = 0.026 for left), superior temporal pole (P = 0.001 for right and P = 0.03 for left) bilaterally, and right parahippocampus, middle temporal pole (P = 0.02), and superior temporal gyrus (P = 0.034) in AD.

Diffusion tensor imaging findings

Patients with MCI demonstrated significantly reduced FA in the left APV (P = 0.03) and increased MD in genu of CC (P = 0.04) relative to controls. Abbreviations elaborated in Supplementary Table 2. Patients with AD relative to controls had significantly reduced FA in bilateral APV (P = 0.014 for left and P = 0.007 for right) and splenium (P = 0.001), with a corresponding increase in MD values over bilateral APV (P = 0.02), left TWM genu (P = 0.007), and splenium (P < 0.001). A direct comparison of patients revealed significantly reduced FA in bilateral PPV (P = 0.001) and APV (P = 0.04 for left and P = 0.05 for right) as well as increased MD in bilateral APV (P = 0.02 for left and P = 0.04 for right), splenium (P = 0.03), and right hippocampus (0.048) in AD relative to MCI.

^{1}H MR spectroscopic findings

Patients with MCI have significantly lower NAA/mI (P = 0.005) and higher Cho/Cr (P = 0.045) ratios than controls [Supplementary Table 3]. Patients with AD showed significantly lower NAA/mI (P < 0.001) along with higher mI/Cr (P = 0.002) and Cho/Cr (P = 0.014) ratios. Comparison between patients with MCI and those with AD revealed significantly lower NAA/mI (P = 0.002) and higher mI/Cr (P < 0.001) in AD relative to MCI.

Multimodal classification based on T1-weighted magnetic resonance imaging, diffusion tensor imaging, and Proton magnetic resonance spectroscopy

We tested the performance of our multimodal classification method in the identification of MCI from AD and healthy controls. Table 2 summarizes the classification accuracy of our multimodal combination method, compared with individual modalities. ROC analysis revealed that the combined measurements of structural MRI, DTI, and ^{1}H MRS consistently achieve more accurate discrimination between MCI and controls and MCI and AD [Figure 2]. More specifically, for classifying MCI from healthy controls, our multimodal classifier achieved a significant area under the curve (AUC) (0.89, P < 0.001), with 93.9% sensitivity and 70% specificity. While considering an individual modality, the DTI provides the best classifier (AUC [0.798, P < 0.001]) with a sensitivity of 90.9% and specificity of 50%. On the other hand, for classifying MCI from AD, our multimodal classification method revealed the highest overall AUC (0.926, P < 0.001), with 93% sensitivity and 85.6% specificity. In addition, the best AUC (0.854,

Table 1: Comparison of demographic and neuropsychological measures between subjects

	NC	MCI	AD	Bonferroni corrected *P* value		
				MCI versus NC	AD versus NC	AD versus MCI
Demographic						
n	20	33	15			
Sex (male/female)	10/10	23/10	9/6	0.225	0.325	1.00
Mean age (mean±SD in years)	62.27±7.52	69.13±6.00	69.45±5.48	<0.001	0.011	1.00
Education (years)	12.80±3.68	11.29±3.25	12.85±3.64	0.95	0.253	0.08
Cognitive						
ACE	89.32±8.96	81.23±7.67	68.64±9.26	0.009	<0.001	<0.001
RAVLT cumulative learning score	52.80±8.12	37.89±10.1	25.36±7.15	<0.001	<0.001	<0.001
RAVLT 20 min recall score	12.29±1.76	6.54±3.49	2.00±1.73	<0.001	<0.001	<0.001

NC=Normal control, MCI=Mild cognitive impairment, AD=Alzheimer's disease, ACE=Addenbrooke's cognitive examination, RAVLT=Rey Auditory Verbal Learning Test, SD=Standard deviation

Table 2: Receiver operating characteristic results for the performance comparison of voxel-based morphometry, diffusion tensor imaging and proton magnetic resonance spectroscopy, and multimodal combination of these neuroimaging methods

	MCI versus controls			MCI versus AD		
Modality	AUC	Sensitivity (%)	Specificity (%)	AUC	Sensitivity (%)	Specificity (%)
T1 weighted MRI	0.775	78.8	70.0	0.829	90.9	60.6
DTI	0.798	90.9	50.0	0.854	72.7	87.9
^{1}H MRS	0.787	87.9	60.1	0.836	81.8	75.8
Multimodal	0.890	93.9	70.0	0.926	93	85.6

AUC=Area under the curve, DTI=Diffusion tensor imaging, ^{1}H MRS=Proton magnetic resonance spectroscopy, MCI=Mild cognitive impairment, MRI=Magnetic resonance imaging, AD=Alzheimer's disease

$P < 0.001$) on individual modality is obtained for DTI, with sensitivity of 72.7% and high specificity of 87.9%.

Logistic regression revealed that a combination of GM, WM, and ^{1}HMRS provides the best overall fit for predicting the diagnosis of MCI. Our best fit classifier included each variable from each modality with maximum AUC for classifying MCI. The score was derived from regression coefficient weighted sum of three modalities for predicting MCI from controls (7.0* [volume of PCC]-9.9* [MD value of Genu] +0.7* [NAA/mI value of PCC]) and MCI from AD (2.7* [volume of HP] +19* [FA value of PPVL] +1.2* [NAA/mI value of PCC]). The combined approach for MCI control classification achieved an AUC (AUC = 0.88; $P < 0.001$) [Figure 3a] with a cutoff score of 3.12, with 82% sensitivity and 90% specificity: 27 out of 33 MCI patients were correctly classified as MCI and 18 out 20 controls were correctly classified as controls. The combined classifier for MCI-AD achieved a highest AUC (AUC = 0.93; $P < 0.001$) [Figure 3b] with a cutoff score of 23.6, with 88% sensitivity and 91% specificity in which 30 out of 33 MCI patients were correctly classified as MCI and 13 out of 15 AD patients were correctly classified as AD.

Discussion

In this article, we have proposed a statistical classifier using logistic regression to discriminate patients with longitudinally stable amnestic MCI from healthy controls and AD. The diagnosis of AD and MCI in its initial stages is still challenging. Hence, predicting the risk of progression from MCI to AD is extremely relevant for future treatment trials. Although clinical and cognitive tests are used in practice, these are not able to identify the more subtle patterns of the disease process at an early stage, and clinical manifestations on neuropsychology are evident well into disease progression in prodromal dementia. Considering that around 10%–12% of MCI progress annually to overt dementia,[13] doubts have been raised regarding the existence of this entity as a notional one[14] and we attempted to study the morphometric and metabolic signatures of MCI that would aid in objective characterization of stability versus an "at risk" state in what is considered to be a prodromal dementia. This assumes significance in centers where specific biomarkers such as 11C-PiB PET, tau PET, and CSF biomarkers are not readily available.

Many existing studies have used structural MRI for measuring GM density (in the form of voxel maps),[15] volume/shape,[16,17] or cortical thickness. It is well known that multivoxel ^{1}H-MRS of the PCC is sensitive to the biochemical changes during the pathologic progression of AD before there is a significant loss of neuronal integrity commensurate to atrophy of the same region in MCI. Therefore, MRS has proven potential for predicting and monitoring different pathological stages in the course of AD.[18] Researchers have demonstrated that a combination of multiple biomarkers can improve the prognostic ability, but these have combined MRI, CSF, and PET analysis as opposed to multimodal MRI techniques alone.[17,19,20] Many studies have used only structural MRI and specifically, hippocampal volumetry to differentiate AD (or MCI) from controls.[21,22] The limited predefined ROIs considered in many studies constrain sampling of spatial–temporal pattern of structural and functional or metabolic abnormalities in their entirety, and we have tried to offset this limitation using whole-brain VBM and values from multiple areas of WM during DTI analysis. This is pertinent, especially in MCI, wherein accurate prediction of risk of conversion may not be tenable with MRI in isolation, especially in patients in whom hippocampal atrophy has not evolved,[14] and also because MCI/dementia is essentially clinically diagnosed. In addition, for AD classification, there are little differences among accuracy, sensitivity, and specificity for any multimodal classification method, considering the fact that the disease process has already been established, whereas, for MCI classification, the differences are relatively large, for example, a relatively large sensitivity, but low specificity, for each method.[16] This is reflected in our results as well as our study revealed an intermediate measurement in GM density, WM integrity, and metabolite ratio in MCI compared to controls and AD. The utility of a similar combination of MRI biomarkers including structural MRI, DTI, and ^{1}HMRS has not been reported previously from an Indian population.

Our findings on MCI control comparison in relation to PCC and thalamic atrophy are in line with a recent study.[23] However, in contrast to previous studies,[16,24] we could not

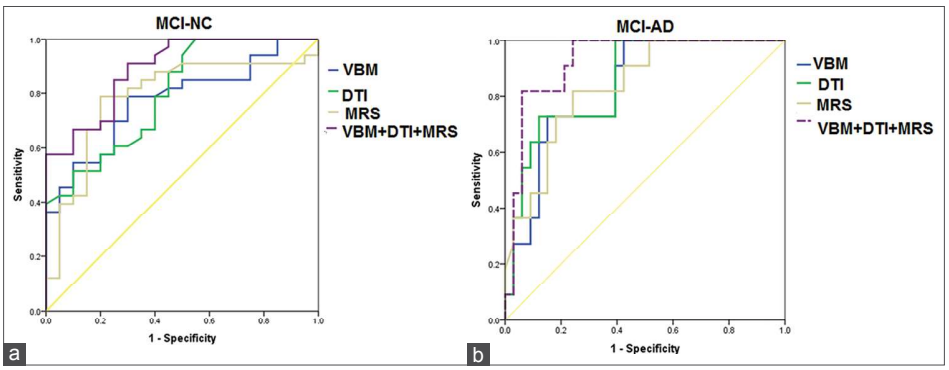

Figure 3: Receiver operator characteristic curves of combined classifiers for classification of (a) mild cognitive impairment from controls and (b) mild cognitive impairment from Alzheimer's disease

find any hippocampal atrophy in amnestic MCI compared to controls, possibly reflective of relative cognitive stability in our MCI. A longer period of follow-up is warranted to conclude on their evolution into multiple domain involvement versus early dementia. Regarding DTI, our observations corroborate with Chen *et al*.,[24] who described DTI changes in APV, PPV, and genu in MCI patients. The major pathological process contributing to reduced anisotropy in the cortical and subcortical WM tracts in AD and MCI may be either due to the presence of subclinical ischemic changes[25] or an increased susceptibility of oligodendrocytes to free radical and other metabolic damages.[26] In addition, there may be alterations in microvasculature, WM rarefaction with axonal damage, and gliosis[27] in brains of patients with AD favoring a mixed pathology beyond amyloid plaques and neurofibrillary tangles. Our finding of reduced posterior DTI indices of WM integrity in MCI and early AD mirrored the GM pathology in posterior brain regions relative to anterior regions.[28,29] The presence of significant regional brain WM anisotropy changes in both MCI and AD groups suggests that posterior regional anisotropy changes in normal-appearing WM of patients with MCI may play a role in the progression toward AD.[30] Furthermore, the anisotropy changes in the splenium of the CC might because of Wallerian degeneration seen in AD pathology.[31,32] The diffusivity changes in the genu of the corpous callosum (CC) in MCI patients might support the retrogenesis hypothesis, as the genu is known to myelinate much later than other WM regions.[33]

The MRS analysis revealed lower NAA/mI levels and higher Cho/Cr levels in the PCC in MCI. The metabolite NAA is a neuronal cell marker, and it can quantify neuronal loss or dysfunction, whereas myoinositol is a glial marker and its activation in MCI patients may be associated with glial activation and inflammation in the pathology of AD.[15]

Among the MRI biomarkers, the most sensitive measurement for discriminating MCI from control was DTI with high sensitivity and only moderate specificity. However, multimodal classifier using genu ADC values, posterior cingulate cortex volume, and NAA/mI MRS ratios produced a discernible improvement in diagnostic classification, with 82% sensitivity and 90% specificity. Considering the accuracy obtained with back classification of the MCI (30/33 correctly classified), it is evident that each modality (volumetry, DTI, and MRS) has its utility in achieving good combinational classification.

Expectedly, for discriminating MCI from AD, the volumetric findings in hippocampus, inferior temporal, and superior temporal pole as well as WM integrity in the periventricular areas showed high sensitivity. Furthermore, the neuronal markers of NAA/mI in the posterior cingulate region discriminated MCI from AD with sensitivity higher than DTI. The reduced NAA levels in AD have been well correlated with the presence of neuritic plaques and neurofibrillary tangles.[34] Moreover, these reductions in NAA/Cr and increases in mI/Cr ratios in the PCC have been demonstrated to be highly linked with the Braak neuropathological stages.[35] Previous findings indicated that NAA/mI ratio has enabled a highest sensitivity (82%–83%) and specificity (80%–95%) for the differentiation of AD from controls.[23,36] Our MRS findings in AD corroborate with another study which demonstrated correlation between reduction of regional glucose metabolism measured by [18F] FDG-PET and NAA/mI by MRS in the PCC of MCI, AD, and healthy controls.[37] A lower NAA/mI for the AD group compared to controls failed to reveal statistical significance in MCI group, unlike our results. Their findings suggest that brain glucose metabolism is a surrogate marker of synaptic activity[38] which should correlate with the measures of neuronal activity and density such as NAA/mI by MRS.

Overall, the utility of our combined classifier using hippocampal volume, FA of the posterior periventricular WM, and adjoining NAA/mI ratio is demonstrable on our back classification accuracy. The current study is limited by certain factors. First, the AD group sample size was small. We, however, primarily attempted to provide a classifier for MCI due to which the AD group served as a "diseased-control" cohort. Second, the other modalities such as CSF, PET, and APOE are not included in the model due to nonavailability of these tests at our center at the time of initiation of this study.

Conclusions

We have introduced a robust method to objectively classify MCI participants in comparison to early AD and cognitively normal healthy controls. We have proposed a new multimodal MRI measure combining cortical GM volume, FA, and MD at the voxel level and metabolite ratio at posterior cingulate region. The discrimination between MCI and AD patients reached a high sensitivity when relevant regions were selected. This result implies that multimodal analysis gives better results than unimodal analysis and hence may be a useful tool to assist in prognostication in MCI. Future studies utilizing our model for prediction on individual patients with stable and unstable MCI are required to gauge its utility over proven non-MRI biomarkers

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Conflicts of interest

There are no conflicts of interest.

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Results: Pathological intraneuronal structures such as dystrophic neurites of senile plaques (DN), neurofibrillary tangles (NFT), neuropil threads, and granulovacuolar degeneration (GVD) showed anti-linear polyubiquitin antibody immunoreactivity. Quantitative analysis revealed that approximately a half of the structures that were immunoreactive for anti-ubiquitin antibody also showed anti-linear polyubiquitin immunoreactivity, however, the positive ratio was variable among the cases.

Conclusion: Our results demonstrate that a subset of ubiquitin-positive pathologic structures that appear in the AD brain also show linear ubiquitin immunoreactivity. The linear ubiquitin chain is thought to play a role in the NF-κB pathway and to promote neuroinflammation. Thus, linear ubiquitination in the neurons of the AD brain may play a role in the pathogenesis of AD.

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2132
WCN17-0168
SHIFT 6 - DEMENTIA
Tachykinin receptor antagonist, N-Acetyl-L-tryptophan alleviates spatial memory deficits in Alzheimer's disease model in rats

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Background: Neuroinflammation and apoptosis plays an important role in the pathophysiology of Alzheimer's disease (AD). Neuropeptide substance P mediated signaling pathway leads to neurogenic inflammation. Whether N-Acetyl L-Tryptophan (NAT), an antagonist of substance P and inhibitor of cytochrome c provides neuroprotection in AD remains unknown.

Objective: The present study investigated role of N-Acetyl-L-Tryptophan in Alzheimer's disease, in aluminium chloride induced cognitive dysfunction model in rats.

Patients and Methods/Material and Methods: Rats were treated with aluminium chloride (10mg/Kg) intraperitoneally for 42 days to induce dementia. After 42 days, two doses of NAT (30mg/Kg and 50mg/Kg) were administered for 28days along with aluminium treatment. Morris water maze test was employed to assess the memory. Acetylcholinesterase activity and antioxidant enzyme levels were measured in hippocampus and striatum. Total cholesterol and glucose levels in serum were also estimated.

Results: Among two dose levels of NAT, treatment with higher dose (50mg/Kg) was found to be effective. We demonstrate that NAT improves acetylcholinesterase activity and antioxidant enzyme level in hippocampus and frontal cortex. Further, impaired glucose homeostasis and lipid levels were corrected by NAT.

Conclusion: We propose that NAT provides neuroprotection due to its ability to block the substance P mediated neuroinflammation, inhibiting free radical generation, anti-apoptotic activity and correcting altered glucose and lipid levels in periphery.

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2133
WCN17-2609
SHIFT 6 - DEMENTIA
A pilot study on mapping structural and functional connectivity in early Alzheimer's disease(AD) in comparison to stable mild cognitive impairment (MCI) and healthy controls

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Background: There is a paucity of studies that address the utility of whole brain functional and structural connectivity analysis in early dementia.

Objective: To study differences in resting state functional MRI (rsfMRI) determined functional connectivity and tractography-estimated structural connectivity in early AD relative to controls and cognitively stable MCI.

Patients and Methods/Material and Methods: rsfMRI and 3D T1-weighted anatomical images were acquired using 3 Tesla scanner on 15 early AD (CDR≤1), 19 longitudinally-stable MCI (single-domain-18) and 10 controls. Group level independent component analysis was used to derive rsFMRI networks. Dual regression was performed on the coherent networks and threshold free cluster enhancement were implemented with a significance of $p<0.05$ (family-wise-error correction). Grey-matter volumes were analyzed by voxel-based-morphometry (VBM) and the structural integrity was analyzed by Tract-Based-Spatial-Statistics (TBSS).

Results: No significant differences were noted in structural and functional connectivity between MCI and controls. The early AD group compared to controls and MCI revealed decreased functional connectivity changes in default-mode network precuneus, supracalcarine cortex) with reduced anterior connectivity (Figure.1) and in visual networks (precuneus; lateral occipital cortex; lingual gyrus).

The whole brain TBSS analysis found significant structural changes in forceps minor, forceps major, superior and inferior longitudinal fasciculus, inferior fronto-occipital fasciculus in AD compared to controls (Figure.2.) and MCI.

VBM-based atrophic clusters are depicted in (Figure.3)

Conclusion: Precuneus being the seat of visual attention and encoding, forms the most important seed for functional connectivity mapping in classifying early AD and sub-classifying MCI due to AD. Tractography

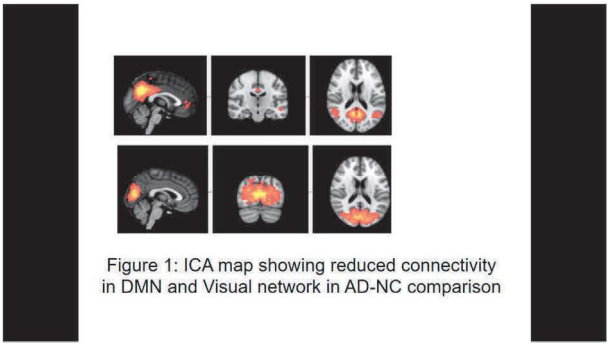

Figure 1: ICA map showing reduced connectivity in DMN and Visual network in AD-NC comparison

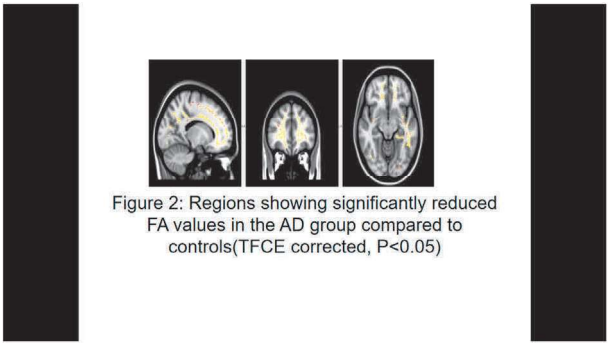

Figure 2: Regions showing significantly reduced FA values in the AD group compared to controls(TFCE corrected, P<0.05)

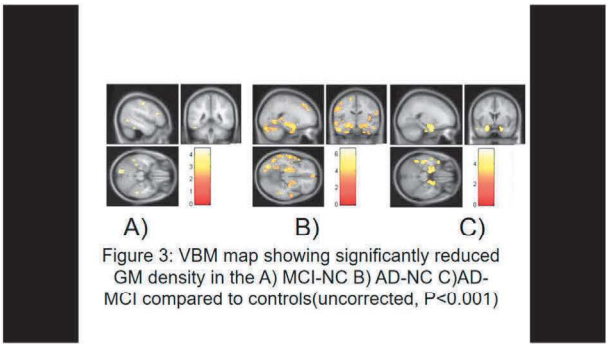

Figure 3: VBM map showing significantly reduced GM density in the A) MCI-NC B) AD-NC C)AD-MCI compared to controls(uncorrected, P<0.001)

changes are indicative of structural network impairment independent of volumetric differences between AD and MCI.

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WCN17-3282

SHIFT 6 - DEMENTIA

Z score - a novel FDG pet score to assess hypo/hypermetabolism in Alzheimers disease

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Background: Alzheimer's disease is the most common dementia and it is imperative to differentiate it from other causes of dementia. We present Z score analysis in FDG PET which demonstrates a signature pattern in AD.

Objective: To assess Z scores in FDG-PET in AD patients and to evaluate its utility in assessing hypo/hypermetabolism in different gyri of the brain.

Patients and Methods/Material and Methods: 8 patients were included in the study. All patients were diagnosed to have AD based on clinical history, neurological examination, cognitive assessment – MOCA, Addenbrookes, clinical dementia index. FDG- PET was performed for all of them. After processing the mapped image, grey scale images are color coded based on standard deviation Z score. Reconstructed images were presented for analysis using 3D stereotactic surface projection (SSP) and compared with CT imaging. Analytical software used is Cortex ID, GE healthcare. Negative Z scores indicated hypermetabolism and positive Z scores indicated hypometabolism.

Results: It was observed that Z scores was elevated in bilateral caudate nucleus in all patients, bilateral posterior cingulate, anterior cingulate, frontal association cortex in 7 out of 8 patients, bilateral temporal and parietal association cortex in 6 out of 8 patients. Z scores were negative in visual cortex, cerebellum, vermis and pons in 7 out of 8 patients suggesting sparing of these areas in AD.

Conclusion: Z scores are invaluable markers of hypo/hypermetabolism and its importance is highlighted here to understand the signature pattern of uptake in AD. Z Scores are extremely useful for differentiating AD from other types of dementia.

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2135

WCN17-2200

SHIFT 6 - DEMENTIA

Disease modifying efficacy of memantine in Alzheimer's disease; a pooled analysis of 13 randomized controlled trials

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Background: Memantine is an FDA approved symptomatic treatment for Alzheimer's disease (AD). However, its disease modifying efficacy remains controversial.

Objective: The aim of this meta-analysis is to synthesize evidence from published randomized controlled trials (RCTs) about the disease modifying efficacy of memantine in AD patients.

Patients and Methods/Material and Methods: A computer literature search of PubMed, EBSCO, and CENTRAL was conducted. Studies were screened and data of relevant RCTs were extracted and analyzed using Review Manager software (Cochrane collaboration – version 5.3). Efficacy measures (listed in Figure 1) were pooled as standardized mean difference (SMD) in the random effect model meta-analysis described by Dersimonian and Laird.

Efficacy Measure	Abbreviation
Clinician's Interview-Based Impression of Change Plus Caregiver Input	CIBIC-Plus
Severe Impairment Battery	SIB
Alzheimer's Disease Assessment Scale-cognitive subscale	ADAS-cog
Alzheimer's Disease Cooperative Study Activities of Daily Living	ADCS-ADL
Mini-Mental State Exam	MMSE
Neuropsychiatric Inventory	NPI

Figure 1. Shows the efficacy measures and their abbreviations.

Results: Data from 13 RCTs (n = 3635) were analyzed. The overall SMD of change in CIBIC-plus favored the memantine over the placebo group for patients with mild to moderate AD and those with moderate to severe AD (SMD -0.17 and -0.28, respectively). In terms of MMSE, NPI, ADAS-cog, and SIB, the overall SMD of change from baseline to end point did not favor the memantine or control group. The Memantine group showed better ADCS-ADL scores in patients with moderate to severe AD (SMD 0.17, 95% CI [0.08 to 0.27]) but not for those with mild to moderate AD (SMD -0.03, 95% CI [-0.15 to 0.10]). The incidence of agitation was 21% less in AD patients taking memantine compared to those receiving placebo (RR 0.79, 95% CI [0.64 to 0.99]).

Conclusion: Current evidence does not support a disease modifying efficacy of memantine in AD patients.

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Differentiating mild cognitive impairment from normal cognition and frank dementia utilizing structural changes observed on magnetic resonance imaging

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Full Text

Mild cognitive impairment (MCI) is a transitional phase between normal cognitive function and dementia. MCI is basically diagnosed when the cognitive score is between 1 and 2% of the lower value of age- and sex-matched normative data. MCI may often progress leading to dementia, may remain static, or may improve. The diagnosis of MCI and early dementia depends on the age- and sex-matched application of neuropsychological scales. MCI are of various subtypes, including the amnestic, the non-amnestic and the multidomain types. Particularly, the amnestic variety usually progresses to Alzheimer dementia. A community study in Eastern India reveals that the prevalence of MCI in India is around 14.89%.[1]

In comparison to the MCI, the diagnosis of dementia depends on the structural (magnetic resonance imaging) or functional imaging modalities (single photon emission computed tomography [SPECT] or positron emission tomography [PET]), which reveals metabolic changes and hypoperfusion in the parieto- temporal association cortex.[2]

Addition of the two softwares provide novel information that is very helpful in establishing the diagnosis of MCI: The voxel based morphometry (VBM) helps to find out the presence of generalized brain grey matter atrophy; and, the automated regional volumetry (ARV) helps to assess the extent of regional brain atrophy in both subjects suffering from MCI and dementia.[3]

The aim of the present study by Sheela Kumari et al., was to quantify the structural changes in cognitively stable patients suffering from MCI and AD (Alzheimer dementia) compared to the individuals with no cognitive impairment, employing the quantitative volumetric magnetic voxel based resonance imaging (vMRI) analytic tools such as ARV and VBM.[4]

The AD subjects revealed widespread cerebral atrophy in bilateral parahippocampi, amygdale, fusiform gyri, superior and middle temporal poles, inferior temporal gyri, and medial orbitofrontal regions, along with atrophy in the right precuneus and left angular regions indicating temporal lobe, frontal lobe and parietal lobe involvement.

Gray matter (GM) volumes in MCI assessed using the automated regional volumetry showed significant differences for the left hippocampus, right precuneus, and bilateral superior temporal gyri when compared to the individuals with normal cognition. Also, in patients with AD, bilateral hippocampi, precunei, superior temporal gyri, parahippocampi, right amygdala, and right superior temporal pole revealed significant grey matter (GM) volume loss when compared to that seen in controls.

Considering the MCI patients, GM volume loss was detected in the superior temporal gyrus and left hippocampus, and variably over the mesial brain regions (cuneus, precuneus, and the cingulate cortex), in comparison to individuals with normal cognition. Although the observation of hippocampal atrophy has been documented to be a marker of future progression in MCI, atrophy of the precuneus, a part of the parietal lobe, suggests the involvement of the posteromedial regions of the cortex in MCI, and in addition, to atrophy of the temporal lobe. Thus, ARV suggests a far wider involvement of the anterior and posterior brain substance, signifying a faster disease progression that may often lead to a frank dementia.

Another aspect of this study was correlation of these structural findings revealed on MRI imaging parameters with the neuropsychological assessment scales and their scores.[4] The correlation obtained between the cognitive scores and the regional volume loss was very specific in the included patients with MCI and AD.

Thus, the assessment of patients with MCI utilizing the new software is very useful and can predict the progression of the cognitive impairment from stage of MCI to frank dementia. However, this study needs validation with a longitudinal cognitive evaluation for about 5 to 6 years, since around 10-15% patients with MCI would progress to Alzheimer's dementia, correlating with the neuroimaging findings.

For patients with MCI, the accurate prediction of the probability of their progression to AD is important from the aspect of patient care; it shall also enable the identification of participants in the clinical trial who will deteriorate rapidly in their cognitive functions, and thus, provide a setting to enable an early intervention. Biomarkers based on neuroimaging modalities could offer complementary information regarding different aspects of disease progression.[5]

This study is well conducted and the authors have made adequate attempts to assess the preliminary results in correlating neuroimaging finding in Indian subjects with MCI with those suffering from dementia as well as with cognitively normal subjects. We agree that the study needs further validation utilizing the 3 Tesla magnetic resonance scanner, by carrying out more cross-sectional and longitudinal studies from different parts of India, considering the multiethnic character of the Indian population.

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MR volumetry in mild cognitive impairment (MCI) – A useful marker to predict progression

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Full Text

Mild cognitive impairment (MCI) is an intermediate stage between age associated cognitive impairment and dementia.[1] In general, a person with cognitive complaints, and the findings of its impairment on testing but with normal functioning, will be characterized as having MCI. It can be classified based on the underlying cognitive deficit (amnestic or non-amnestic) or the number of domains affected (single versus multiple). MCI is a heterogeneous condition with several causes like depression, medications, alcohol use, and chronic medical diseases that need to be ruled out.[1] Though the concept of MCI has generated controversy, it is a useful clinical construct because it helps clinicians communicate the diagnosis to patients who have cognitive impairment but do not meet the criteria for dementia. In addition, MCI is a target for disease-modifying agents. It enables an early intervention in order to prevent or delay the development of dementia.

MCI estimates are about 12-18% among older adults above the age of 60 years.[1] The annual progression rate/year is around 10% though there is wide variation based on population or specialist memory clinic data. MCI can also remain stable or improve but it is important to note that even in studies where it appeared to improve, most patients ultimately progressed to dementia.[2] The amnestic form (aMCI), in particular, has been considered an early stage of Alzheimer's disease (AD).[1]

There are two important issues in the approach to a patient with MCI in the clinic. One is the way to identify patients who are at an increased risk of dementia. Sheelakumari and colleagues have compared 24 patients with aMCI, 13 with early AD and 25 healthy controls using voxel-based morphometry (VBM) and automated regional volumetry (ARV) techniques to quantify the gray matter volumes on magnetic resonance imaging (MRI) of the brain.[3] All subjects also underwent Addenbrooke's cognitive examination (ACE) and Rey Auditory Verbal Learning Test (RAVLT), which have been adapted to the local population. The aMCI group was stable; the group did not have patients progressing to AD over a study period of 1.5 years. The major findings in this study were that the aMCI group had a predominant atrophy in the superior temporal gyrus, left hippocampus and mesial

frontoparietal regions while the AD group showed more extensive atrophy in the limbic region (especially the parahippocampal gyrus, bilateral hippocampi and amygdala), temporal neocortex and precuneus. Atrophy measures were significant compared to controls for both the patient groups. The AD group showed more regional atrophy than the MCI group but these differences were not significant. Only the overall gray matter atrophy was significant between the two groups. Memory learning and recall correlated with left hippocampal volume in MCI and with right temporal neocortex in AD patients. Quantitative MR imaging in MCI patients using VBM is a novel observation for India. These MR imaging techniques need infrastructure, trained personnel and are time consuming. Most studies in the West have used these quantitative MR techniques for more than a decade for identifying the imaging markers of MCI and preclinical AD. Some studies have shown that visual rating using a standardized scale, which is quicker and simpler, seems to perform nearly as well.[4] A prospective study following MCI patients with imaging and neuropsychological testing to identify the markers indicating the progression to dementia would be useful in our setting.

Use of robust memory tests adapted to the language and practices of the local population added strength to the study. The major drawback, which the authors themselves point out, is the short duration of 1.5 years over which they designated the aMCI as being stable or non-progressive. This duration appears inadequate and most studies report a minimum of 3-5 years. It is surprising that MCI patients in this study had a relatively long duration of symptoms (5.8 ± 3.7 years; range 2.1-9.5 years). The authors do not mention comorbid conditions and also anxiety, though they ruled out depression, which may have contributed to the symptoms. It is also interesting that the memory measures correlated with the left hippocampus as expected in MCI, but unexpectedly with the right temporal cortex in the AD group. Verbal memory measures are expected to correlate with the left side. This is a surprising finding, which has not been discussed. In a meta-analysis of VBM studies from 429 aMCI patients, of whom 142 converted to AD, the gray matter volumetric reduction in the medial temporal lobe (left hippocampus and parahippocampal gyrus) was the consistent biomarker to predict conversion from aMCI to AD.[5] The National Institute of Aging and Alzheimer's Association have also formulated criteria for diagnosing MCI using biomarkers. For instance, the stable MCI patients in this study have an intermediate probability of belonging to the AD group, as they had atrophy in the mesial temporal region, considered a neuronal marker of injury.[6]

The relationship between depression and MCI is complicated. Depression may be seen in 25-40% patients with MCI.[1] Depression is associated with an increased risk of MCI in normal elderly subjects, and also in the case when MCI is associated with an increased risk of progression to dementia. It was customary to exclude depression in clinical trials of MCI but doing so greatly reduces the sensitivity of diagnosis of AD.[7]

The second important issue in dealing with a patient with MCI would be to reverse cognitive impairment if possible, or to delay the conversion rate to dementia. Treating depression and other comorbid medical conditions, addressing vascular risk factors, withdrawing medications causing cognitive impairment, indulging in cognitive activities, and being engaged with life and physical exercise are some of the strategies in the management of MCI. Only the last factor, physical exercise, has been shown to be beneficial in studies [8] but it is a good clinical practice to follow the other measures as well. We should direct our efforts to diagnosing patients early, suggesting interventions and in having a close follow up. Initiating cholinesterase inhibitors in MCI patients is a poor clinical practice and there is no evidence to support it.

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SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY, TRIVANDRUM

STATEMENT OF EXPENDITURE

1. ***Sanction Order No and Date*** : SR/CSI/90/2012 (G) Dated 21/11/2013
2. ***Total Project Cost*** : Rs. 18,77,600/-
3. ***Revised Project Cost (if applicable)*** : Rs. 23,09,600/-
4. ***Date of Commencement*** : 21/11/2013
5. ***Grant received in each year***:
   - a. ***1st Year*** : Rs 9,39,200.00
   - b. ***2nd Year*** : Rs 6,00,000.00
   - c. ***3rd Year*** : Rs 6,50,000.00
   - d. ***Interest, if any*** : Nil
   - e. ***Total (a+b+c+d)*** **: Rs 21,89,200.00**

6. Statement of Expenditure: (Month wise expenditure incurred during the current financial year)

Month & year	Expenditure incurred/committed
21/11/2013 to 31/03/2014	0.00
01/04/2014 to 31/03/2015	Rs 1,97,254.00
01/04/2015 to 31/03/2016	Rs 5,03,299.00
01/04/2016 to 31/03/2017	Rs 6,52,484.00
01/04/2017 to 31/03/2018	Rs 5,73,213.00
01/04/2018 to 22/09/2018	Rs.2,62,950.00

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY, TRIVANDRUM

STATEMENT OF EXPENDITURE

(For the Period from 01/04/2014 to 22/09/2018)

SLNo (I)	Sanctioned Heads (II)	Funds allocated (Indicate sanctioned or revised) (III)	Expenditure Incurred					Total Expenditure IV + V + VI+VII+VIII (IX)	Balance as on 22/09/2018 III- IX (X)	Requirement of Funds up to 31nd March, 2019	Remarks (if any)
			1st Year (1st April, 2014 to 31st March, 2015) (IV)	2nd Year (1st April, 2015 to 31st March, 2016 (V)	3rd Year (1st April 2016 to 31st March 2017) (VI)	4th Year (1st April 2017 to 31st March 2018 (VII)	5th Year (1st April 2018 to 22nd Sept 2018 (VIII)				
1.	Equipment	450000	141512	126965	0.00	43196	0.00	311673	138327	-	-
2.	Fellowship	1209200	0.00	212516	510003	157536	172000	1052055	157145	-	-
3.	Consumables	300000	5742	94258	80775	308220	83473	572468	(-) 272468	-	-
4.	Contingencies	40000	0.00	40000	11706	14261	7477	73444	(-) 33444	-	-
5.	Travel	40000	0.00	29560	0.00	0.00	0.00	29560	10440	-	-
6.	Overhead	150000	50000	0.00	50000	50000	0.00	150000	0.00	-	-
7.	Total	**2189200**	**197254**	**503299**	**652484**	**573213**	**262950**	**2189200**	**0.00**	-	-

Name and Signature of Principal Investigator:
Date: 18/12/18

Signature of Competent financial authority: 18/12/2018
(With seal) Date:

N. VENKITA SUBRAMANIA IYER
Sr. ACCOUNTS OFFICER
Sree Chitra Tirunal Institute for
Medical Sciences and Technology
Thiruvananthapuram

*** DOS – Date of Start of Project**

Note :

1. Expenditure under the sanctioned heads, at any point of time, should not exceed funds allocated under that head, without prior approval of DST i.e. Figures in Column (VIII) should not exceed corresponding figures in Column (III)

Utilisation Certificate for each financial year ending 31st March has to be enclosed along with request for carry-forward permission to the next financial

FINAL CONSOLIDATED UTILISATION CERTIFICATE

(For the Financial Year/Period ending 22/09/2018)

1.Title of the project/scheme:	The human brain mapping project – a resting state fMRI of health controls and patients with mild cognitive impairment (MCI) & degenerative dementia of the Alzheimer`s type (AD)
2. Name of the Organization	Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum
3. Principal Investigator	Dr. Ram Shekhar N Menon, Additional Professor, Dept. of Neurology, SCTIMST
4. Dept. Of Science and Technology Date of sanctioning the project	**NO.SR/CSI/90/2012 (G) dated 21/11/2013**
5. Amount brought forward from the previous financial year quoting DST letter No. & date in which the authority to carry forward the said amount was given	` 0.00
6. Amount received from DST during the financial years 2013-14 to 2017-18 *(Please give No. and dates of sanction orders showing the amounts paid)*	` 21,89,200/-
7. Other receipts/interest earned, if any, on the DST grants	**NIL**
8. Total amount that was available for expenditure during the financial years	` 21,89,200/-
9. Actual expenditure (excluding commitments) incurred during the financial year **01/04/2014 – 22/09/2018** (statement of expenditure is enclosed)	` 21,89,200/-
10. Unspent balance refunded, if any (Please give NIL details of cheque No. etc.)	**NIL**
11. Balance amount available at the end of the financial year 31/03/2016	` 0.00
12. Amount allowed to be carried forward to the next financial year	` 0.00

Certified that out of **` 2189200.00 (Rupees Twenty One Lakh Eighty Nine Thousand Two Hundred only)** of grants in aid sanctioned during the years 2013-114 to 2017-18 in favor of Director, SCTIMST vide DST Order No: **SR/CSI/90/2012 (G)** dated **21/11/2013 and ` 0.00 (Rupees ZERO only)** on account of unspent balance of the previous year, a sum of **` 2189200.00 (Rupees Twenty One Lakh Eighty Nine Thousand Two Hundred only)** mentioned against col. 9 has been utilized on the project / scheme **"The human brain mapping project – a resting state fMRI of health controls and patients with mild cognitive impairment (MCI) & degenerative dementia of the Alzheimer`s type (AD)"** for the purpose for which it was sanctioned and that the balance of **` 0.00 (Rupees ZERO only)** remaining unutilized at the end of the year ~~will be adjusted towards the grants-in-aid payable during the financial year 2016-17~~

Sr. Accounts Officer-A
Date:
N. VENKITA SUBRAMANIA IYER
Sr. ACCOUNTS OFFICER
Sree Chitra Tirunal Institute for
Medical Sciences and Technology
Thiruvananthapuram
18/12/18

Principal Investigator
Date: 18/12/18

DIRECTOR
SCTIMST
19 DEC 2018
निदेशक / Director
श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान
Sree Chitra Tirunal Institute for
Medical Sciences and Technology
त्रिवेन्द्रम / Trivandrum-695011

(To be filled by DST)

Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled / are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Project files are subject to annual C & AG Audit in SCTIMST

UC id – 18212990 & 18212991