MCI Patients Declining and Not-Declining at Mid-Term Follow-Up: FDG-PET Findings

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Abstract: Patients with Mild Cognitive Impairment (MCI) not converted to dementia at one to three years follow-up represent an heterogeneous group across studies, by including 'late converters' but also patients without any neurodegenerative disease. We tested the hypothesis that the combination of memory and brain metabolic assessment could identify subgroups of memory decliners (MCI/Decl) and non-decliners (MCI/noDecl) before a long follow-up time is available. From twenty-nine patients with amnestic MCI (aMCI) at baseline, three groups were identified at follow-up: 10 patients who converted to AD (MCI/AD); 10 patients either showing episodic memory worsening or reaching the floor effect on memory and declining in other key tests (MCI/Decl) and 9 patients showing no memory worsening or even improvement (MCI/noDecl). They were compared with a group of fourteen elderly controls (CTR) by means of basal FDG-PET voxel-based analysis (SPM2). Two hypometabolic clusters were found in MCI/AD versus CTR, including the bilateral posterior cingulate cortex, the left parietal precuneus and the left fusiform gyrus. MCI/AD showed also a large hypometabolic region, mainly including the left medium and superior temporal gyri and inferior parietal lobule, when compared to MCI/noDecl. The MCI/Decl showed a hypometabolic region in the left medial temporal lobe versus both CTR (hippocampus) and MCI/noDecl (parahippocampal gyrus and hippocampus). No significant difference was found in the comparison between CTR and MCI/noDecl, neither in the comparison between MCI/Decl and MCI/AD. Thus, non converter MCI patients comprised a sub-group of 'decliners' with AD-like metabolic and cognitive patterns, likely including 'late converters', and a sub-group lacking this pattern, with stable or improving memory function and a brain metabolic picture similar to that in healthy controls. Combining neuropsychological and FDG-PET information could be used for prognostic purposes in aMCI patients at medium-term follow-up.

Keywords: FDG-PET, episodic memory, amnestic mild cognitive impairment, Alzheimer's disease.

INTRODUCTION

Data from brain metabolism, as assessed by means of ^{18}F-fluorodesoxyglucose Positron Emission Tomography (FDG-PET), are recognized as one of the 'biomarkers' to evaluate the pathophysiological changes of Alzheimer's disease (AD) at a pre-dementia stage [1]. During the progression of the disease from mild cognitive impairment (MCI) to the early stages of AD, a consistent number of PET studies have shown hypometabolism in some crucial regions, namely the medial temporal lobe (MTL), the posterior cingulate, the precuneus, and the associative posterior temporo-parietal cortex, with some topographic differences [2-7]. More recently, hypometabolism in lateral temporal and frontal cortex has been reported as well [8, 9].

Clinical evaluation of MCI is carried out by neuropsychological and neuropsychiatric assessment and there is a general consensus about episodic memory impairment being the earliest cognitive deficit of prodromal AD (amnestic MCI: aMCI). Of great interest for the implications on possible therapeutic interventions is the finding that episodic memory changes have been found to occur more than five years prior to the clinical onset of dementia of the AD type [10, 11].

FDG-PET data has been analysed to pick up the peculiar features of those MCI patients who would have developed AD ('converters') versus those MCI who would not ('non converters'). In the majority of the studies, investigators have been looking for factors at baseline FDG-PET able to discriminate between converters and non-converters and to predict the course of the disease [2-6, 8]. Only in a few investigations a second FDG-PET has been performed at the time of AD conversion [2].

In all such studies, the group of non converters has been variously defined, as 'non converters' or 'stable' MCI. However, because the follow-up time is generally limited to a few years, the brain metabolic pattern of the converters is actu-

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ally that of 'early converters', while the pattern of 'late converters' is not known at this stage, because they are mixed, within the 'non converter' group, together with patients without apparent neurodegeneration and with subtle functional changes.

Therefore, the so called group of 'stable MCI' (or 'non converter MCI') actually comprises a really stable (or even improving) MCI sub-group ('non decliners') and a sub-group that continues to decline, although not fulfilling the criteria for dementia ('decliners') as yet. For this reason, it is still unclear whether MCI can be considered as a transitional period before development of AD or whether there is a categorical difference between the two conditions, implying that some patients will never convert to AD but follow a more benign clinical course [12].

The heterogeneity of symptoms, biomarkers and patients' inclusion and exclusion criteria across FDG-PET studies may have affected the comparisons between non converter MCI patients and both controls and converters, by including 'decliners' and 'non decliners' together in the non converter group and accounting for the large variability in the reported conversion rates. Hence, peculiar patterns in the topography of FDG-PET changes in the aMCI patients who continue to decline may lead to an earlier diagnosis of AD, even if the clinico-neuropsychological follow-up data does not fulfil the diagnosis of dementia as yet.

'Decliners' might show a distinct hypometabolic pattern, either different or similar -but milder- as compared to early converters. In the latter case, these patients may be suspected to be 'late converters', and thus either in an earlier stage of AD or with more grey matter and/or a larger functional brain reserve than early converters [12, 13].

To investigate the brain metabolic heterogeneity of non converter aMCI, we followed over time a group of patients undergoing FDG-PET at the time of the first evaluation. At the last clinico-neuropsychological follow-up visit, aMCI patients were re-classified into AD, aMCI decliners and aMCI non-decliners. The two latter groups were identified on the basis of scores on the delayed recall measure of an episodic verbal memory test, the neuropsychological hallmark of AD. Baseline FDG-PET data was compared among these patient groups as well as with a group of elderly healthy controls by voxel-based analysis.

METHODS

Patients

The study included outpatients with memory complaints in whom an objective memory deficit was demonstrated at baseline by means of neuropsychological tests. Dementia was excluded on the basis of a clinical interview with the patient and caregiver, using the Mini-Mental State Examination (MMSE) [14] for general cognition as well as the questionnaires for the Activities of Daily Living (ADL) [15], instrumental (IADL) [16], and Clinical Dementia Rating (CDR) scale (0.5 in all patients).

Patients underwent a standard battery of blood count, blood chemical examinations and urinanalysis, according to the commonly followed rules to exclude secondary causes of cognitive impairment. Presence of analphabetism, major vision disturbances, psychiatric illnesses, epilepsy, major head trauma, Parkinsonism, previous stroke or TIA, and brain masses were other exclusion criteria. A mild depressive trait, as ascertained by the 15-item Geriatric Depression Scale (GDS), was not an exclusion criteria. Neuropsychiatric symptoms were assessed by interviewing the informant with the Neuropsychiatric Inventory (NPI) [17]. Patients scoring higher than 0 on the delusion and the hallucination NPI items were excluded. MRI was performed in all patients by means of a 1.5 Tesla equipment. Only patients with MRI evidence of major stroke were excluded, while white matter hyperintensities, leukoaraiosis and lacunae were not exclusion criteria. The modified Hachinski ischemic scale [18] was ≤ 2 in all patients.

The initial group comprised thirty-six aMCI patients (22 females, 14 males; mean age: 76.0 ± 5.5 years). These patients underwent a neuropsychological battery, including evaluation of i) verbal episodic memory (immediate and delayed recall, IR and DR from now on) by the 6-trial Selective Reminding Test (SRT) [19], ii) visuomotor abilities, divided and attentional shifting by the Trail-Making Test, forms A (TMT-A) and B (TMT-B), iii) categorical verbal fluency (2' test for animals), iv) visuoconstructional abilities by a test involving copying figures, including simple copy and copying with guiding landmarks of the Mental Deterioration Battery [20], v) abstract and logical reasoning by the Raven's PM38 matrices (set A-D, according to Spinnler and Tognoni [21]), vi) executive attention by the Stroop color-word test (correct items achieved in 30 secs., according to Barbarotto *et al.* [22]). The clock completion test (CCT, as evaluated according to Watson *et al.* [23]) was used as a mixed measure of visuospatial abilities and executive functions and the Symbol-Digit test [24] as a mixed measure of working memory and executive functions.

A Z-score lower than -1.5, computed on the normative database of each test and corrected for age and education, was established for impairment in a specific cognitive domain. According to the Petersen's criteria [25], patients with a Z-score lower than -1.5 either on the IR or DR of the SRT (single-domain aMCI) as well as patients scoring less than -1.5 both on SRT and in other cognitive domains (multi-domain aMCI) were considered.

All patients were carefully treated for systemic comorbidity; drugs known to depress brain synaptic transmission, such as benzodiazepines and tricyclic antidepressants, were withdrawn. Then, patients commenced follow-up with a clinical examination (also including MMSE, ADL and IADL questionnaires, and CDR) every 6 months and with both clinical and neuropsychological examination on a yearly basis. During follow-up visits, the same neuropsychological protocol as at baseline was applied.

The follow-up time ranged from 1 to 3 years (Table **1**). During the follow-up period, two patients developed frontotemporal dementia or dementia with Lewy bodies, according to the current criteria [26, 27], after 1 and 2 years respectively, and were excluded. In five patients clinical information was available that excluded dementia, however they did not complete or refused to undergo neuropsychology, thus they were not being considered in the present analysis

Accordingly, the final study group included 29 patients (Table **1**). During the follow-up, ten among the 29 patients developed dementia of the AD type (MCI/AD), according to the NINCDS-ADRDA [28] and DSM-IV criteria. The mean annual conversion rate to all dementia was approximately 19% (12/36=33% in 20.4 months mean follow-up time); the mean annual conversion rate to AD was approximately 16% (10/36=28% in 20.4 months mean follow-up time).

Since no definite rule has yet been accepted to define worsening in such a context and several neuropsychological tests failed to predict conversion to dementia [29], in order to subgroup the patients we chose the score of the delayed recall on the verbal episodic memory test (SRT) whose decrease is regarded as the hallmark of AD [1, 30] and we arbitrarily included in the group of decliners (MCI/Decl) those patients scoring 0 at follow-up examination (independently of their score at basal examination) and those losing at least 0.5 in Z-score between baseline and follow-up examinations. In six patients a severe memory deficit was highlighted by a DR score of 0 already at baseline and confirmed at follow-up, thus showing the well known 'floor effect' of DR in AD pathology. They were included in the MCI/Decl group, although further decline cannot be established in a strict sense on the basis of DR score. However, in these 6 patients further cognitive deterioration between baseline and follow-up was confirmed by a worsening score on at least two among the MMSE, as a global cognitive measure, the digit symbol and the CCT (the last two tests showing significant changes, besides the DR score, between baseline and follow-up in MCI patients). On the other hand, the patients with a score higher than 0 at follow-up examination who had remained stable (< 0.5 Z-score worsening) or even improved, were included in the non-decliners group (MCI/noDecl). In no instance a worsening of two or more among the MMSE, the digit symbol and the CCT was observed in MCI/noDecl patients.

According to this classification, the MCI/Decl group included 10 patients and the MCI/noDecl group included 9 patients. Tables **1**, **2** report the main demographic, clinical and neuropsychological characteristics of the three groups of MCI/AD, MCI/Decl, and MCI/noDecl.

Controls

The protocol received the approval of the local Ethics Committee. Control subjects were healthy volunteers giving their informed consent, recruited during University courses dedicated to elderly people. Their healthy condition was carefully checked by means of general medical history, clinical examination and the same exclusion criteria as for patients, with the exception of cognitive complaints. MMSE was performed and only subjects with a normal score (i. e. $\geq$ 26) were considered. Moreover, only subjects with a CDR of 0 were included. Fourteen subjects matched these requisites and were included (Table **1**). The subjects underwent the same neuropsychological battery as patients and brain MRI (all but 4 who underwent CT because of metallic devices, n=2, or claustrophobia, n=2).

^{18}F-FDG PET

PET was performed within 3 months from the baseline clinical-neuropsychological examination (mean: 29.9 days in patients and 29.8 days in CTR). Subjects fasted for at least six hours. Before radiopharmaceutical injection, blood glucose was checked and was < 7.8 mmol/l in all cases. After a 10 min. rest in a silent and obscured room, with eyes closed and ears unplugged, subjects were injected with approximately 370 MBq of ^{18}F-FDG *via* a venous cannula, according to the guidelines of the European Association of Nuclear Medicine [31]. They remained in the room for 30 min. after the injection, they were then moved to the PET room where scanning started approximately 45 min. after the injection and lasted another 20 min. A polycarbonate head holder was used to reduce head movements during the scan. Images were acquired by a 'Discovery ST' PET-CT equipment (GE Healthcare, USA) on a 128x128x64 matrix (isotropic voxel of 2.34 mm) in 2-dimensional mode with a total axial field of view of 15 cm and no interplane gap space. Images were reconstructed by a OSEM algorithm, 16 subset and 2 iterations. Dicom files were exported and converted to Analyse files.

Statistics

ANOVA and ANOVA for repeated measures was applied to assess the statistical significance of differences in demographics and neuropsychological test scores among groups.

Using SPM2 (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 6.5 (Mathworks, Natick, Massachusset, USA), PET data were subjected to affine and non-linear spatial normalization into the standard Talairach and Tournoux's space The spatially normalized set of images were then smoothed with a 8 mm isotropic Gaussian filter to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. The resulting statistical parametric maps, SPM{t}, were transformed into the unit of normal distribution (SPM{z}).

Correction of SPM coordinates to match the Talairach coordinates was achieved by the subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging). SPM t-maps were thresholded using a $p<0.001$ at voxel level, uncorrected for multiple comparisons. Considering the relatively low sensitivity of PET without repeated measures and the explorative nature of the study, a more conservative threshold could lead to false negative result [32, 33].

The significance of identified regions was established at a $p<0.05$ and corrected for multiple comparisons at the cluster level. Only clusters containing more than 100 voxels were considered to be significant. At voxel level, the false discovery rate (FDR) and the family-wise error (FWE) methods for multiple comparison correction were also explored. Comparisons were performed by means of the 'compare populations: 1 scan/subject (Ancova)' option, with age, gender and education as 'nuisance' variables. The CTR group was compared with the three groups of patients, namely MCI/AD, MCI/Decl, and MCI/noDecl. Moreover, the MCI/Decl group was compared to either the MCI/AD group or the MCI/noDecl group, and the MCI/noDecl group to the MCI/AD group.

RESULTS

Group Characteristics

Controls were slightly younger than the patient groups and a gender inbalance was found in MCI/noDecl group as

Table 1. Main Demographic and Clinical Characteristics of the Three Patient Groups and Controls

	Group			
	MCI/AD	**MCI/Decliners**	**MCI/Non Decliners**	**Controls**
N	10	10	9	14
Age (years.)	77.7 ± 4.8	75.7 ± 5.3	75.8 ± 5.9	70.6 ± 6.4*
Sex M/F	2/8	4/6	5/4*	4/10
Education (years)	8.8 ± 3.9	8.0 ± 3.9	11.0 ± 5.3	11.2 ± 4.5
Blood glucose level (mmol/l)	4.67±0.56	4.88±0.61	4.78±0.69	4.68±0.49
Apo E 3/4 genotype carriers #	5/8	3/8	3/7	n. a.
Hachinski §	0.80+0.79	0.70+0.67	0.78+0.67	n. a.
NPI	7.5 ± 9.5	7.9 ± 8.7	10.9 ± 11.5	7.3 ± 7.0
Follow-up time (months)	22.0 ± 13.1	19.5 ± 9.3	23.7 ± 12.8	
Baseline MMSE	27.5 ± 1.4	27.2 ± 2.3	27.0 ± 2.0	29.0 ± 1.1*
MMSE at follow-up	24.0 ± 2.6*	26.5 ± 3.1	27.1 ± 2.9	

GDS= 15-item Geriatric Depression Scale; NPI= Neuropsychiatric Inventory; MMSE= Mini-Mental State Examination;
MCI= Mild Cognitive Impairment; n.a. = not available; # = data not available in all patients; § modified Hachinski ischemic scale.
* = $p<0.05$ in comparison with the other groups (ANOVA)

Table 2. Mean (± Standard Deviations) of the Neuropsychological Test Scores (Corrected for Age and Years of Education) in the Three Patient Groups and Controls

	GROUPS						
	MCI/AD		**MCI/Decliners**		**MCI/Non Decliners**		**Controls**
	Baseline	**F.U.**	**Baseline**	**F.U.**	**Baseline**	**F.U.**	**Baseline**
CCT	2.9 ± 2.7*****	3.1 ± 3.1*****	1.3 ± 2.2	2.6 ± 3.1*** §**	3.6 ± 3.0*****	1.9 ± 2.7 **§**	1.2 ± 1.9
SRT IR	28.7 ± 2.4*****	25.4 ± 6.1*****	28.7 ± 6.7*****	25.9 ± 6.1*****	32.0 ± 3.7*****	32.8 ± 7.2*****	47.6 ± 9.4
SRT DR	1.4 ± 1.1*****	0.2 ± 0.5*** §**	0.8 ± 1.1*****	0.5 ± 0.6*****	2.3 ± 1.2*****	4.3 ± 1.7 *** §**	6.8 ± 2.2
Verbal Fluency	16.9 ± 4.3*****	9.5 ± 4.7*** §**	17.5 ± 6.7*****	16.7 ± 6.3*****	18.9 ± 3.0*****	15.2 ± 4.2*****	27.2 ± 5.4
TMT A (secs)	60.9 ± 12.1*****	77.8 ± 18.4*****	65.8 ± 13.5*****	65.6 ± 12.3*****	70.3 ± 33.8*****	79.0 ± 32.4*****	45.9 ± 12.8
TMT B (secs)	241.3 ± 94.7*****	283.7 ± 178.1*****	217.8 ± 114.0*****	206.8 ± 85.9*****	223.7 ± 154.0*****	241.3 ± 135.2*****	132.0 ± 51.4
Figure copying: simple	8.9 ± 0.7	8.5 ± 0.8	9.0 ± 1.2	8.9 ± 0.9	8.1 ± 2.0	7.8 ± 1.0	8.9 ± 1.9
Figure copying: with guiding landmarks	68.0 ± 1.9	60.2 ± 5.4*** §**	64.2 ± 4.9	61.4 ± 6.4*****	62.1 ± 7.8	58.9 ± 8.5*****	65.6 ± 4.0
Stroop color	33.9 ± 7.1	31.5 ± 7.8*****	35.8 ± 6.5	30.4 ± 9.2*****	32.8 ± 5.8*****	28.6 ± 7.6*****	39.0 ± 7.8
Stroop color-word	12.3 ± 4.2*****	8.1 ± 4.3*** §**	11.2 ± 6.1*****	11.3 ± 7.9*****	12.1 ± 4.6*****	8.4 ± 5.6*****	16.9 ± 5.0
Raven's PM 38	26.2 ± 9.0	20.6 ± 8.8*****	28.7 ± 6.8	27.8 ± 6.5	25.9 ± 8.7	23.1 ± 7.7*****	30.5 ± 8.9
Digit symbol	24.7 ± 1.9*****	19.4 ± 4.7*** §**	28.7 ± 12.7*****	23.6 ± 9.4*** §**	23.1 ± 9.3*****	20.4 ± 9.0*****	37.1 ± 9.0

CCT= Clock completion test (a mixed measure of visuospatial abilities and executive functions); SRT-IR = Selective Reminding Test-Immediate Recall; SRT-DR = Selective Reminding Test-Delayed Recall, exploring immediate and long-term verbal episodic memory; TMT A = TrailMaking test form A; TMT B = Trailmaking test form B, exploring executive functions; Raven's PM 38 = Raven's 38 Progressive Matrices, exploring abstract and logical reasoning. Figure copying assesses visuoconstruction abilities, the Stroop test is a measure of executive attention, while the Digit symbol test is a mixed measure of working memory and executive functions. As for CCT, TMT A and TMT B higher values indicate a worse performance, while for all the other tests higher values indicate a better performance.
* = $p<0.05$ statistical significance of difference between either baseline or follow-up values between patient groups and controls (ANOVA).
§ = $p<0.05$ statistical significance of difference between follow-up and baseline values within the same group (ANOVA for repeated measures); the asterisk is shown near the value at follow-up.

compared to the other groups. Therefore, both age and gender along with education were taken into account as confounding variables in the voxel-based PET comparisons. As expected, the baseline MMSE score was significantly higher in CTR than in the three patient groups, whereas at follow-up the MCI/AD group showed a lower MMSE score than both MCI/Decl and MCI/noDecl (Table **1**).

Both at baseline and at follow-up examinations, the majority of neuropsychological test scores were significantly lower in the three patient groups than in CTR.

ANOVA for repeated measures showed a significant worsening of DR, verbal fluency, constructional praxis with guiding landmarks, Stroop color-word and digit symbol test scores in MCI/AD, a significant worsening of the Clock completion and digit symbol test scores in MCI/Decl, and a significant improvement of the Clock completion test and DR scores in MCI/noDecl (Table **2**).

PET Comparisons: CTR Group Versus Patient Groups

Two hypometabolic clusters were found in MCI/AD versus CTR, including the bilateral posterior cingulate cortex, the left parietal precuneus, and the left fusiform gyrus (Fig. (**1**); Table **3**). The MCI/Decl showed a hypometabolic region in the left medial temporal lobe versus CTR, including the hippocampus (Fig. (**2**); Table **3**).

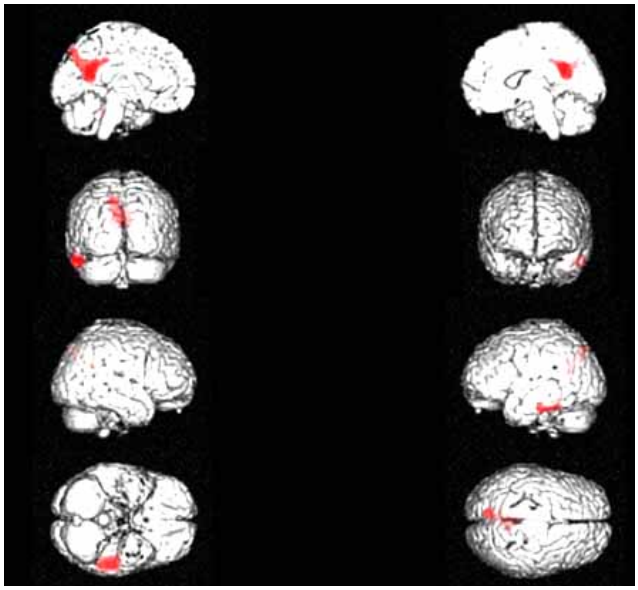

Fig. (1). Statistical parametric maps showing the regions of significant decrease metabolism in MCI/AD patients compared with controls, obtained by SPM2 analysis.

This comparison did not reach the significance level when corrected for multiple comparisons, at cluster level (p=0.095), but it was significant (p=0.019) at cluster level without correction and at voxel level even applying either FWE (p=0.008) or FDR (0.021) corrections. Moreover, the Z score of maximum at voxel level was the highest among all of the comparisons (Table **3**). Since the location of this latter cluster was very close to that found to be significant in the MCI/Decl *versus* MCI/noDecl comparison (Fig. (**3**); Table **3**) and meaningful from a pathophysiological standpoint, we judge it to be of clinical relevance.

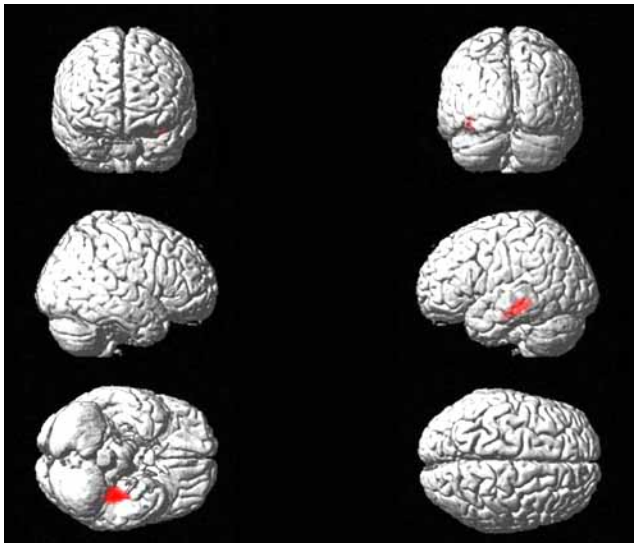

Fig. (2). Statistical parametric maps showing the regions of significant decrease in metabolism in MCI/Decl patients compared with controls, obtained by SPM2 analysis.

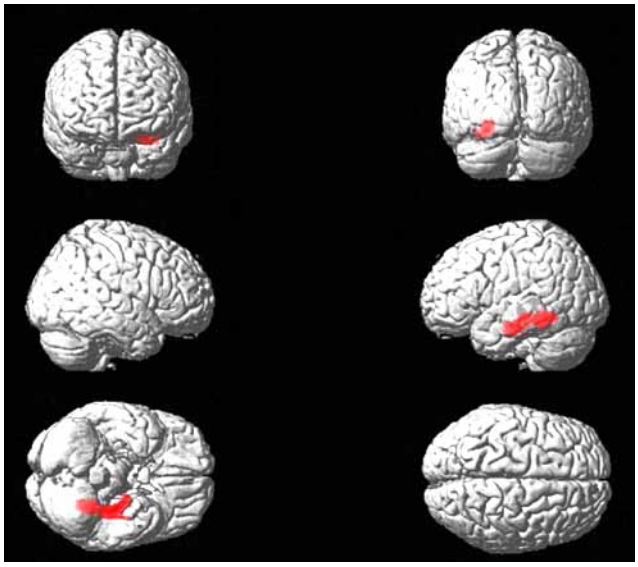

Fig. (3). Statistical parametric maps showing the regions of significant decrease in metabolism in MCI/Decl patients compared with MCI/noDecl, obtained by SPM2 analysis.

No significant difference was found in the comparison between CTR and MCI/noDecl.

PET Comparisons Among Patient Groups

MCI/Decl showed a hypometabolic area in the left parahippocampal gyrus and hippocampus as compared to MCI/noDecl (Fig. (**3**); Table **3**), but no significant difference

Table 3. Numerical Results of SPM Comparisons (Uncorrected Height Threshold p<0.001 at Voxel Level)*

Comparison	Cluster level			Voxel level			
	Cluster extent	Corrected P value	Cortical region	Z score of maximum	Talairach coordinates	Cortical region	BA
CTR- MCI/AD	372	0.05	L temporal	4.44	-50, -36, -23	fusiform gy.	20
	1064	0.001	L limbic	3.80	-6, -53, 19	posterior cingulate	30
			L parietal	3.75	-8, -70, 44	precuneus	7
			R limbic	3.58	10, -49, 23	posterior cingulate	31
CTR- MCI/Decl	309	0.095	L temporal	5.08	-36, -26, -10	hippocampus	
MCI/noDecl- MCI/Decl	573	0.027	L temporal	3.62	-26, -45, -8	parahippocampal gy.	37
				3.53	-26, -49, -6	parahippocampal gy.	19
				3.52	-32, -28, -10	hippocampus	
MCI/noDecl – MCI/AD	3771	0.000	L temporal	4.91	-60, -58, 0	Medium temporal gy.	37
			L parietal	3.80	-52, -64, 7	Inferior parietal lobule	40
			L temporal	3.57	-59, -53, 19	Superior temporal gy.	22

*A value of P≤0.05, corrected for multiple comparison at cluster level, was accepted as statistically significant. In the 'cluster level' section on left, the number of voxels, the corrected P value of significance and the cortical region where the voxel is found, are all reported for each significant cluster. In the 'voxel level' section, all of the coordinates of the correlation sites (with the Z score of the maximum correlation point), the corresponding cortical region and BA are reported for each significant cluster. L, left; R, right; BA, Brodmann's area. In the case that the maximum correlation is achieved outside the grey matter, the nearest grey matter is indicated with the corresponding BA.

as compared to MCI/AD. On the other hand, a large significant hypometabolic cluster was found in the MCI/AD group versus MCI/noDecl group, mainly including the left medium and superior temporal gyri and the inferior parietal lobule (Table **3**).

DISCUSSION

The present study shows that a group of aMCI patients not converted to dementia during an average follow-up period of about 20 months actually included heterogeneous subjects. In those who did not worsen in terms of episodic memory, brain metabolic pictures at baseline were not statistically different from that in healthy controls. On the other hand, in those who substantially worsened or reached the floor effect on the delayed verbal recall test, brain metabolic distribution at baseline was not statistically different from that in aMCI patients who will have developed AD dementia, which is in keeping with previous studies reporting that AD-like metabolic patterns in MCI predict conversion to dementia within several years [34].

Episodic memory is recognized as the first cognitive deficit in the early stages of AD and there is evidence that this impairment correlates with grey matter loss in MTL, spreading to the posterior association cortex when converting to dementia. Moreover, low initial DR scores were found to significantly correlate with greater decline at follow-up [35] and to predict conversion to dementia [36, 37]. Our patient grouping included in the MCI/Decl group some aMCI patients reaching the floor effect in the DR measure already at baseline, and thus not worsening in memory in a strict sense. However, having a score of 0 on DR both at baseline and at follow-up qualifies these patients as the most severely affected for memory among the non converter MCI patients, and thus including them in the MCI/Decl group seemed reasonable. Moreover, cognitive deterioration in these patients was confirmed by a worsening score on at least two among the MMSE, the digit symbol and the clock completion test, which was never the case in any of the MCI/noDecl patients.

Recent investigations have reported a better diagnostic accuracy in predicting dementia when FDG-PET is combined with neuropsychological assessment, reaching sensitivities and specificities comparable to those achieved after long-term follow-up [4, 9, 38]. In the present study, the DR score at medium-term follow-up was used to sub-group non converter MCI patients and to test the hypothesis whether FDG-PET at baseline would significantly discriminate subgroups. Although the subtypes of MCI could have different metabolic patterns on FDG-PET [39] single and multi-domain amnestic MCI were analysed together due to the limited number of patients, whose further distinction would have produced groups too small for meaningful statistical analyses.

The group of MCI/Decl showed relative hypometabolism in the left MTL in comparison to both MCI/noDecl and healthy controls. Although a longer follow-up time is needed, and we really do not know the fate of these patients as yet, we speculate that most of the MCI/Decl patients eventually developing dementia in the following years (late converters), may share the same severity of memory deficit and a similar brain metabolic pattern with those who are already converted to AD (early converters). This assumption is also supported by the lack of significant metabolic differences between MCI/Decl and MCI/AD in the present study. The early decrease of hippocampal and enthorinal cortex volume

in AD [40-43], beginning long before the symptoms appear, is in line with this hypothesis, although a linear time and topographic progression of the disease has not yet been established.

Several studies have found hypometabolism in the posterior cingulate cortex to be the earliest functional change in mild AD [2, 8, 44, 45], possibly due to its tight functional connections with the MTL [46] and to the evidence that FDG is mainly a measure of synaptic activity [47]. However, by improving anatomical localisation tools, metabolic reduction was shown in the hippocampus already at a MCI stage [48, 49]. In this respect our *a-priori* choice of smoothing images with a 8 mm Gaussian isotropic filter during the normalization step in SPM, along with the high resolution and the lower filtering of the PET data obtained by means of a last generation equipment, may have enhanced the capability of Voxel Based Analysis to identify subtle MTL changes at group level, a finding which is generally better highlighted in individual scans following coregistration with MRI volumetric scans.

The notion of the heterogeneity of the MCI population at both neuropsychological and functional level can be of value in treatment planning. When effective disease-modifying drugs will be available, the MCI/Decl patients could be selected for the more invasive CSF sampling and more time-consuming MTL segmentation at MRI, to confirm the AD-like pathophysiological pattern and to start neuroprotective treatment.

The finding that in a substantial part of aMCI patients non converted to dementia (37% in this study) the DR scores on the SRT improved during the follow-up, after providing adequate treatment with supportive and general measures, and that an AD-like brain metabolic pattern could not be demonstrated, are at least as important as the finding of early hypometabolism in MCI/Decl. As a prognostic source of information to the patient him/herself and to the family, this is in fact of paramount relevance resulting in reduction of anxiety and more straightforward caregiving tasks. Moreover, expensive and potentially dangerous pharmacological treatment can be avoided.

As compared with CTR, MCI/AD showed relative hypometabolism in the left MTL, the bilateral posterior cingulate and the left precuneus, while MCI/Decl showed only left MTL hypometabolism. Moreover, MCI/AD also showed relative hypometabolism in a large cluster, mainly including the medium and superior temporal gyri and inferior parietal lobule in the left hemisphere as compared to MCI/noDecl group. We believe the core features of our data was to show the similarities between MCI/AD and MCI/Decl and the substantial differences between these two groups and both CTR and MCI/noDecl, with hypometabolic clusters of difference found in some of the main reported critical regions in AD process. Though it is the case in the majority of these studies, the small number of subjects in each group did not allow us to go deeper into the discussion about the role of each area during progression to AD.

In fact, there is still much debate on the brain region earlier affected by hypometabolism in the natural history of AD, whether the MTL or the posterior cingulate/precuneus, but such a debate risks being meaningless, until large patient samples followed over a long time become available. This limitation is shared by the present study as well, due to the objective difficulties in selecting, PET-scanning and following-up aMCI patients in a single-centre study.

The group selection produced a significantly younger control group as compared to the three patient groups. Despite age was considered as a nuisance variables in all PET comparisons, thus correcting for the most of the age effect on brain metabolic patterns, some residual age effect cannot be excluded. Moreover, the slight prevalence of males in the MCI/noDecl group is likely to be the result of chance, due to the limited number of subjects in each group. However, gender was taken into account in the SPM analyses, thus ruling out the most of its effect on comparisons.

In conclusion, combined episodic verbal memory and FDG-PET assessment in aMCI patients may help to identify a subgroup of patients who are not converted to dementia as yet, but share similar features with aMCI patients already converted to dementia. The time course of AD takes several years from the first memory deficit to overt dementia and the groups referred to above may well represent late and early converters, respectively. Verification of the final outcome requires longer follow-up studies in large cohorts of patients to ascertain whether this assumption is confirmed.

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