Brain metabolic patterns in patients with Suspected non-Alzheimer's pathophysiology (SNAP) and Alzheimer's disease (AD): is [^{18}F] FDG a specific biomarker in these patients?

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Short title: FDG-PET in AD and SNAP

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Abstract

Aim: The present study was aimed to compare the pattern of the brain [^{18}F] FDG uptake in Suspected non-Alzheimer's pathophysiology (SNAP), AD and healthy controls by using 2-Deoxy-2-[18F]fluoroglucose ([^{18}F] FDG) Positron Emission Tomography imaging. Cerebrospinal fluid (CSF) biomarkers amyloid-β1–42 peptide (Aβ1–42) and Tau were used in order to differentiate AD from SNAP.

Materials and methods: the study included 43 newly-diagnosed AD patients (female=23; male=20) according to the NINCDS-ADRDA criteria and 15 SNAP patients (female=12; male =3) and a group of 34 healthy subjects that served as the control group (CG) found to be normal at neurological evaluation (males=20; females=14). A neuropsychological battery was administrated in AD and SNAP subjects; cerebrospinal fluid assay was conducted in both AD and SNAP as well. Brain PET/CT acquisition was started 30 ± 5 min after [^{18}F] FDG injection in all the subjects. Statistical parametric mapping 12 (SPM12) implemented in Matlab 2018a was used for the analysis of PET scans in this study.

Results: As compared to SNAP, AD subjects showed a significant hypometabolism in a wide cortical area that involves the right frontal, parietal and temporal lobes. As compared to CG, AD subjects showed a significant reduction of [^{18}F] FDG uptake in the parietal, limbic and frontal

cortex while a more limited reduction of [^{18}F] FDG uptake in the same areas was obtained when comparing SNAP to CG.

Conclusions: SNAP subjects show a milder impairment of brain [^{18}F] FDG uptake as compared to AD. The partial overlap of the metabolic pattern between SNAP and AD limits the use of [^{18}F] FDG PET/CT in effectively discriminating these clinical entities.

Abbreviations:

2-Deoxy-2-[18F]fluoroglucose : [^{18}F] FDG

Positron emission tomography: PET

Computed tomography: CT

Cerebrospinal fluid: CSF

Total Tau: t-Tau

Phosphorylated Tau: p-Tau

Keywords: [^{18}F] FDG; Alzheimer, SNAP; PET/CT;biomarkers

1. Introduction

Suspected non-Alzheimer's pathophysiology (SNAP) describes a clinical entity where older adults with or without subtle cognitive decline show one of the markers of neurodegeneration (e.g. neuronal injury markers), but test negative for brain amyloid (Aβ) pathology [i.e. cerebrospinal fluid (CSF) assay and positron emission tomography (PET)] and have not been diagnosed with a specific neurodegenerative disorder [1]. The correct identification of SNAP is crucial since it has been reported to affect up to 23% of cognitively healthy adult individuals [1, 2] and is characterized by a benign clinical course with only a minor proportion of patients progressing to mild cognitive impairment (MCI) or clinical AD [2].

In the era of Aβ imaging, the exclusion of one of the pathological hallmarks of Alzheimer's disease (AD) is feasible. Several radiolabeled compounds have been developed for the in vivo visualization of the Aβ burden in brain tissue by means of PET [3]. On one hand, Aβ imaging with PET allows the correct classification of AD and the identification of those subject with MCI that will progress to AD[4]; on the other hand, it represents a suboptimal biomarker in the assessment

of dementia severity[5]. Interestingly, cognitive decline is only weakly related to change in Aβ burden and, most importantly, Aβ deposition increases slowly from cognitive normality to moderate severity[5]. **On the other side, PET with 2-Deoxy-2-[18F]fluoroglucose ([^{18}F] FDG) can efficiently demonstrate significant differences in the brain [^{18}F] FDG uptake when comparing AD with other types of dementia (as frontotemporal dementia, FTD); in subjects affected by MCI the pattern is less specific and reflect the neuropsychological profile, but an involvement of the cingulate cortex and hippocampus is usually observed [6]**. Although extensively studied in AD, less is known about the role of PET imaging in SNAP individuals. To date, most of the studies in SNAP patients have been carried out using amyloid PET tracers, with the main scope to exclude AD as a cause of cognitive impairment in these subjects [1, 2, 7]. To the best of our knowledge, the [^{18}F] FDG pattern has been evaluated only in one previous study, on a limited number of SNAP subjects [8].

The aim of the present study was to compare the pattern of the brain [^{18}F] FDG uptake in SNAP, AD, and healthy controls by using [^{18}F] FDG PET imaging. CSF amyloid-β1–42 (Aβ1–42) was used as a marker of amyloid and CSF tau was used as a marker of neuronal injury, in order to differentiate AD from SNAP.

2. Materials and methods

Cognitive evaluation

A neuropsychological battery (Table 1) was administrated in AD and SNAP subjects and included: Mini-Mental State Examination (MMSE) [9]; verbal episodic long-term memory (Rey Auditory Verbal Learning Test, long-term memory, 15-word list immediate and 15-min delayed recall) [10]; visuospatial abilities and visuospatial episodic long-term memory (Rey Complex Figure Test, copy and 10-min delayed recall) [11]; executive functions (Phonological Word Fluency Test) [12], and analogic reasoning (Raven's Colored Progressive Matrices) [12]. Italian normative data were used in all tests for both score adjustment (gender, age, and education) and to define the cut-off score of normality, determined as the lower limit of the 95% tolerance interval (normative data are reported in the corresponding references).

CSF sampling

CSF sampling in AD and SNAP was conducted with the same modalities reported previously in another study of our group[13]. After lumbar puncture, the first 12 ml of CSF were collected in a polypropylene tube and directly transported to the local laboratory for centrifugation at 2,000 g at +4°C for 10 min. The supernatant was pipetted off, gently stirred and mixed to avoid potential gradient effects, and aliquoted in 1-ml portions in polypropylene tubes that were stored at −80°C pending biochemical analyses, without being thawed and re-frozen. In the AD patients, CSF t-Tau and phosphorylated Tau (p-Tau, Thr181) concentrations were determined using a sandwich ELISA (Innotest® hTAU-Ag, Innogenetics, Gent, Belgium). CSF AB1-42 levels were determined using a sandwich ELISA [Innotest β-amyloid(1-42), Innogenetics] specifically constructed to measure AB containing both the first and 42nd amino acid.

PET/CT scanning protocol

The study has been conducted in the Nuclear Medicine facility of Policlinico Tor Vergata of Rome. The system used was General Electric VCT PET/CT scanner. All the subjects were injected intravenously with [^{18}F] FDG (dose range 185-295 MegaBequerels) and hydrated with 500 ml of saline (0.9 % sodium chloride). PET/CT acquisition was started 30 ± 5 min after [^{18}F] FDG injection and lasted 10 minutes in all the subjects. Reconstructions parameters were as follow: Ordered subsets expectation maximization, 4 subsets and 14 iterations; matrix 256x256; full width at half maximum (FWHM): 5 mm[13, 14]. The system PICASO (www.picaso-project.eu) was used for the share of medical data.

AD and SNAP patients

The present study was conducted on 43 newly-diagnosed AD patients according to the NINCDS-ADRDA criteria [15] and 15 SNAP patients. A general overview of the study population is provided in Table 1.

AD patients were recruited after a detailed clinical assessment including a thorough medical history, neurological examination, and laboratory testing according to a standardized protocol as described in other studies by our group [14, 16].

Selection of SNAP subjects was performed on the basis of CSF parameters as reported in previous studies, among clinically normal participants aged > 65 years [17-19]. SNAP group was defined by the absence of CSF amyloid marker and presence of CSF neuronal injury marker, with or without subtle cognitive decline. The cutoffs for abnormality were less than 450 pg/mL for Aβ1–42, greater than 350 pg/mL for t-Tau, and greater than 50 pg/mL for p-Tau[20]. All SNAP patients

had abnormal t-tau or p-tau in the presence of normal Aβ1–42, regardless of episodic memory ability.

All the subjects examined underwent structural magnetic resonance imaging (MRI) performed within 1 month prior to [^{18}F] FDG PET/CT brain scan. A co-registration of PET and MRI data was carried out in doubtful cases. As for AD, subjects with the isolated deficit and/or unmodified Mini-Mental State Examination (MMSE) = 25/30 during revisits, with a Hachinsky scale and radiological evidence of sub-cortical lesions were excluded from this study. Other exclusion criteria were predefined as follows: patients with other neurological symptoms as dysfunction in the hypothalamus and/or appendices suprasphenoidalis disease; the presence of pyramidal and/or extrapyramidal signs at the neurological examination; patients with thyroid diseases, diabetes, cancer, HIV, or previous brain injury. Table 1 summarizes the main demographic and neuropsychological features of AD and SNAP subjects, as well as CSF amyloid and neuronal injury markers. Groups were matched for sex and age. AD patients did not differ from SNAP patients on neuropsychological tests investigating global cognitive functions (i.e. MMSE), visuospatial abilities, executive functions, and analogic reasoning. However, AD patients displayed statistically significantly lower mean scores in verbal episodic long-term memory than SNAP patients. Finally, as expected on the basis of predefined inclusion and exclusion criteria, significant differences between groups were found in CSF amyloid and neuronal injury markers. AD patients showed higher levels of Aβ1-42 and lower levels of t-tau and p-tau in the CSF than SNAP patients.

CG subjects

Thirty-four chemotherapy-naïve subjects (males, 20; females, 14; mean age, 71 ± 8 years) undergoing an [^{18}F] FDG PET/CT and found to be completely negative for various diseases were

enrolled in the study and served as the control group (CG). The population used as CG has been selected from a population that has been already evaluated in another report from our group[21], representing an optimal match for AD and SNAP patients. Data of amyloid and neuro injury biomarkers among CG are available is a part of them (13 subjects, 38%, see Table 1). Before their inclusion in our study, all of them had previously been evaluated for the absence of clinical signs of AD by an experienced neurologist (A.M.), and the MRI, performed 7 ± 2 days before PET/CT examination, was negative for brain injury in all of them. Participants with the previous history of neurological or psychiatric disorders, use of typical and atypical antipsychotics, sensorial or motor impairments or other clinical conditions which may influence the cognitive performance (such as hypothyroidism or B12 vitamin depletion), history of alcohol or other substance abuse were excluded from the present study.

Informed consent was obtained from all of the patients and CG subjects and procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008[22]. **The study protocol was considered as observational by the internal review board of the Local Ethical Committee of Policlinico Tor Vergata that gave the approval.**

Statistical analysis

Statistical parametric mapping 12 (SPM12) implemented in Matlab 2018a was used for the analysis of PET scans in this study (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). PET data were converted from DICOM to Nifti format using Mricron software available at https://www.nitrc.org/projects/mricron and then subjected to a normalization process. A bias regularization was applied (0.0001) in order to limits biases due to smooth, spatially varying artifacts that modulate the intensity of the image and that can impede the automating processing

of the images. FWHM of Gaussian smoothness of bias (to prevent the algorithm from trying to model out intensity variation due to different tissue types) was set at 60mm cutoff; tissue probability map implemented in SPM12 was used (TPM.nii). A mutual information affine registration with the tissue probability maps[23] was used to achieve approximate alignment to ICBM space template – European brains[24, 25]. Warping regularization was set with the following 1 by 5 array (0,0.001,0.5,0.05,0.2); smoothness (to cope with functional anatomical variability that is not compensated by spatial normalization and to improve the signal to noise ratio) was set at 5 mm; sampling distance (that encodes the approximate distance between sampled points when estimating the model parameters) was set at 3.

We applied an 8-mm isotropic Gaussian filter to blur the individual variations (especially gyral variations) and to increase the signal-to-noise ratio. We used the following parameters and post-processing tools before regression analysis was applied: global normalization (that escalates images to a global value)= 50 (using proportional scaling); masking threshold (that helps to identify voxels with an acceptable signal in them) was set to 0.8; transformation tool of statistical parametric maps into normal distribution; correction of SPM coordinates to match the Talairach coordinates, subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging). Brodmann areas (BA) were identified at a range from 0 to 3 mm from the corrected Talairach coordinates of the SPM output isocenter by using a Talairach client available at http://www.talairach.org/index.html. As proposed by Bennett et al.[26], SPM t-maps have been corrected for multiple comparisons with the false discovery rate ($P \leq 0.05$) and corrected for multiple comparisons at the cluster level ($P \leq 0.001$). The level of significance was set at 100 ($5 \times 5 \times 5$ voxels, i.e., $11 \times 11 \times 11$ mm) contiguous voxels. The following voxel-based comparisons were assessed: AD versus SNAP and vice versa; AD vs CG and vice versa; SNAP vs CG and vice

versa. All the comparisons were performed using a 'two-sample t-test' design model available in SPM12 [16]. We used sex and MMSE and CSF as covariates in the analyses between AD and SNAP; age, sex, and MMSE in the comparisons among AD, SNAP, and CG.

In order to investigate any hemispheric asymmetry of [^{18}F] FDG brain uptake in SNAP subjects, a selected ROI was placed on the cortical gray matter of both hemispheres by means of WFU Pickatlas tool implemented in SPM 12 and further analyzed after a normalization process[27]. The mean signal intensities computed of the whole cluster have been normalized within each subject to the average intensities of the cerebellar Volume of Interest (VOI) as defined by other reports published previously[28, 29]. Data are reported in Table 4.

For the comparison between AD and CG, the voxel-based analysis was performed using a modality adjusted paired t-test (two conditions, one scan/condition) and the following comparison was assessed: AD vs. CG using gender and age as nuisance variables.

Comparisons of [^{18}F] FDG uptake values in different brain regions in SNAP subjects were analyzed with the Mann-Whitney U test (nonparametric test).

Correlation analyses of CSF values with neuropsychological tests results was performed using the nonparametric Spearman correlation.

3. Results

As compared to SNAP, AD subjects showed a significant hypometabolism in a wide cortical area that involves the right frontal, parietal and temporal lobes (Table 2). We did not find any area of increased [^{18}F] FDG uptake when subtracting SNAP to AD subjects. 3D rendering of data presented in Table 2 is shown in Figure 1(a).

As compared to CG, AD subjects showed a significant reduction of [^{18}F] FDG uptake several cortical areas as reported in Table 3, which included parietal, limbic temporal and frontal cortex (Figure 2). A more limited reduction of [^{18}F] FDG uptake was obtained when comparing SNAP to CG (Table 3). For each comparison (Table 2 and Table 3, see below) we reported the Z-score. This value is used in statistics of a value's relationship to the mean (average) of a group of values, measured in terms of standard deviations from the mean.

We did not find any significant difference when comparing [^{18}F] FDG uptake in a selected cortical lobe as compared to an opposite lobe in SNAP subjects (i.e. [^{18}F] FDG uptake in left frontal lobe vs. right frontal lobe etc.). Results are shown in Table 4. Moreover, we did not find differences in [^{18}F] FDG uptake among different lobes of the same hemisphere (i.e. left frontal lobe vs. left temporal lobe) with the exception of the comparisons with the occipital lobe where, as expected, higher values of [^{18}F] FDG uptake were detected (Figure 3).

We did not find any significant relationship between t-Tau values and the results of neuropsychological tests in AD and SNAP subjects. As for p-Tau, we found a significant relationship with Rey Auditory Verbal Learning Test, delayed recall in AD subjects (negative correlation) and Rey Complex Figure Test, copy and Raven's Colored Progressive Matrices in SNAP subjects (negative correlation). We did not find any significant relationship between Aβ1–42 CSF values and neuropsychological assessment in AD and SNAP groups (Table 1).

4. Discussion

SNAP is a biomarker-based concept depicting the presence of AD-like neurodegeneration in individuals without excessive amyloid-β (Aβ) deposition. SNAP was first described in 2012 in a study aimed to evaluate the criteria for preclinical AD proposed by the National Institute on Ageing–Alzheimer disease Association (NIA–AA) [1]. However, it is still a matter of debate whether SNAP should be considered an independent clinical entity with a different biological basis than AD or, conversely, the result of measurement or classification errors [30]. Furthermore, SNAP classification is independent of any particular degree of cognitive impairment. In our cohort, indeed, SNAP individuals were classified regardless of episodic memory ability [14] although, as expected, they showed cognitive impairment. Interestingly, no significant differences were found between AD and SNAP regarding visuospatial abilities, visuospatial episodic long-term memory, executive functions, and analogic reasoning; whereas verbal episodic long-term memory function was more affected in AD than SNAP, suggesting a differential, although partially, in the pattern of impairment. In line with these neuropsychological findings, SNAP patients showed a significant hypometabolism in parietal and limbic cortices than CG subjects, with the involvement of cingulate gyrus and precuneus (Table 3 and Figure 1). These results suggest that SNAP patients show a pattern of hypometabolism similar to that observed in AD patients [31] thus supporting the hypothesis that medial temporal and precuneus tau pathology without amyloidosis might be a major constituent of SNAP [32, 33]. In analogy with a common hypometabolic pattern in AD and SNAP that mainly involves precuneus and cingulate cortex, both conditions showed overlapping p-Tau levels, suggesting that common tau pathology in these areas may account for the clinical similarities observed [30]. According to data reported in Table 1, AD group had higher T-tau in

the CSF and a nearly statistically significant difference in the Rey Complex Figure Test and the Phonological Word Fluency Test compared to the SNAP group. Hence, it could be hypothesized that the AD group could have more extensive cognitive impairment and neuronal injury than the SNAP; this could partially explain the higher extensive [^{18}F] FDG abnormalities in the AD group and the huge difference in the extent of the cluster reported in Table 3. In the SPM analysis, the potential effect of CSF biomarkers is mitigated by the use of Aβ1-42, t-Tau and p-Tau values as covariates.

The regional analysis reported in Table 4 does not show hemispheric differences in [^{18}F] FDG uptake in SNAP patients further suggesting that SNAP condition may include heterogeneous non-AD pathologies. In Figure 3, on the other hand, it is clearly detectable how [^{18}F] FDG uptake is lower in temporal and frontal lobe bilaterally than in the parietal lobes (a pattern that may be consistent with frontotemporal dementia). Even if this difference suggests that SNAP patients may include FTD variant, this finding did not reach the statistical significance and, most importantly, was not confirmed in the comparison with CG (Table 3 and Figure1). In comparison with CG, the parietal lobe, in fact, is one of the cortical areas that show a significant reduction of [^{18}F] FDG uptake ruling out the FTD pattern. It remains to be elucidated the exact pathological process leading SNAP development. In this context, CSF analysis plays still a major role in differentiating SNAP from AD, but represent a highly invasive and costly diagnostic procedure. On the other side, according to our results, [^{18}F] FDG PET does not show major differences in the metabolic patterns observed in AD and SNAP sufficient to consider this diagnostic tool as a candidate in the differential diagnosis between AD from SNAP. Recent studies on amyloid imaging suggest that PET could be determinant for a correct diagnosis in patients with persistent or progressive unexplained MCI, patients with progressive dementia and atypically early age of onset and patients

satisfying core clinical criteria for possible AD because of unclear clinical presentation, either an atypical clinical course or an etiologically mixed presentation[34]. In particular, in the study of Bensaïdane MR et al. [35], the use of amyloid PET resulted in a diagnostic change in 32.1% (17.8% changed from AD to non-AD, 14.3% from non-AD to AD). If one considers the sub-optimal performance of [^{18}F] FDG in our study in discriminating SNAP from AD subjects, it can be concluded that amyloid imaging should be considered instead of [^{18}F] FDG in doubtful cases. Recently, using AD Neuroimaging Initiative (ADNI, http://www.adni-info.org) it has been demonstrated that APOe genotype does not differ significantly between SNAP (negative for amyloid burden and positive for neurodegeneration in imaging biomarker) and subjects negative for both neurodegeneration and amyloid burden, suggesting that APOE and known genetic drivers of AD do not appear to contribute to the neurodegeneration observed in SNAP[36]. In another report from Schreiber S. et al performed on a large population of normal subjects and patients with mild cognitive impairment, SNAP group had a lower proportion of APOE ε4 carriers and less severe abnormalities on neurodegeneration biomarkers associated with AD, such as glucose metabolism[37]. Our results are in agreement with the cited paper of Schreiber S. et al. but data on APOe genotype are available in a limited number of subjects of our study cohort (28 subjects with AD and 5 SNAP subjects). Twelve AD and 2 SNAP had ε4 phenotype; 15 AD and 3 SNAP had the ε3 phenotype and no conclusions can be drawn on the role of APOe genotype on our findings. Despite providing an insightful comparison between AD and SNAP, our study has some limitations to be acknowledged. First, no post-mortem pathological data were obtained in our patients, thus it is not possible to assume that all AD patients had clear amyloid-related pathology. However, biomarker-based validated criteria [12-14] were used. Second, our study was limited by the cross-sectional design making impossible to compare the rate and speed of progression of these

two entities. SNAP remains an interesting model to study the pathophysiology of tau-related degeneration in the absence of detectable amyloid pathology; future, longitudinal studies are mandatory to elucidate the course of metabolic changes in the parietal and limbic regions, and their relationship with cognitive dysfunction. Lastly, also considering that the use of CSF biomarkers could improve the accuracy on in patient's selection in our study, a consensus on cut off values for CSF in AD are still missing; recently it has been suggested that each laboratory must use internally qualified cutoff values and must warrant longitudinal stability in its measurements[38]. The cut-off values used in our study are in line with those proposed by Forlenza OV et al. that have been reported to show acceptable values in terms of sensitivity and specificity for discriminating AD from controls [20]. Nevertheless, cut-off values have to be carefully considered to guarantee the optimal diagnostic performance of biomarkers.

5. Conclusions

SNAP subjects show a milder impairment of brain [18F] FDG uptake as compared to AD. The partial overlap of the metabolic pattern between SNAP and AD limits the use of [^{18}F] FDG PET/CT in effectively discriminating these clinical entities.

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Compliance with Ethical Standards

The authors report no financial disclosures/fundings or conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with 1964 Helsinki declaration and subsequent versions.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Table 1: General overview of the AD and SNAP population examined including cerebrospinal fluid analysis results and neuropsychological evaluation. AD = Alzheimer's disease; SNAP = suspected non-AD pathology.

	AD	SNAP	FDR-corrected P value	CG	Spearman r; P value (AD subjects)	Spearman r; P value (SNAP subjects)
Population	43: f=23; m=20	15: f=12; m=3	0,10	34: f=20; m=14		
Age	70±7 years	75±4 years	0,06	71 ± 8 years		
Aβ1-42 (ng/ml)	347,8±117,52	657,7±125,87	<0,01	818 ± 202,71*		
T-Tau (ng/ml)	789,6±248,55	578,3±163,36	0,01	272 ± 84,23*		
p-tau (ng/ml)	99,7±50,52	79,3±20,03	0,19	40,3 ± 10,93*		
MMSE score	20,0±4,84	21,8±4,55	0,23	28,7 ± 0,84		
Rey Auditory Verbal Learning Test, immediate recall	20,7 ±7,65	30,7±8,46	0,006	33,2 ± 7,57	T-Tau: -0,11; 0,47 P-Tau: 028; 0,06 Aβ1-42:-0,03;0,81	T-Tau: -0,07; 0,57 P-Tau:0,12;0,66 Aβ1-42:0,05;0,85
Rey Auditory Verbal Learning Test, delayed recall	1,9±2,44	4,3±3,74	<0,01	6,6 ± 2,12	T-Tau: -0,02; 0,86 P-Tau: 0,43;<0,01 Aβ1-42:-0,21;0,16	T-Tau: -0,06; 0,82 P-Tau:0,30;0,26 Aβ1-42:0,38;0,15
Rey Complex Figure Test, copy	16,6±9,23	19,4±10,02	0,36	25,3 ± 11,35	T-Tau:-0,08; 0,58 P-Tau:0,13;0,37 Aβ1-42:-0,18;0,23	T-Tau: 0,51; >0,05 P-Tau:0,65;0,01 Aβ1-42:<0,01;0,98
Rey Complex Figure Test, delayed recall	6,9±4,34	9,8±5,75	0,07	9,1 ± 5,24	T-Tau: -0,02; 0,86 P-Tau:-0,08;0,59 Aβ1-42:-0,21;0,16	T-Tau: 0,17; 0,53 P-Tau:0,30;0,26 Aβ1-42:0,10;0,72
Raven's Colored Progressive Matrices	18,9±7,93	20,0±6,89	0,65	24,4 ± 3,62	T-Tau:-0,10; 0,52 P-Tau:-0,17;0,29 Aβ1-42:-0,11;0,48	T-Tau: 0,35; 0,20 P-Tau:0,56;0,03 Aβ1-42:<0,01;0,99
Phonological Word Fluency Test	20,8±8,32	26,8±11,22	0,06	26,9 ± 10,38	T-Tau:-0,20; 0,18 P-Tau:0,15;0,30 Aβ1-42:<0,00;0,93	T-Tau: 0,27; 0,31 P-Tau:0,21;0,43 Aβ1-42:-0,11;0,67

* Data available from 13 subjects (see Chiaravalloti et al. [21])

Table 2. Numerical results of SPM comparisons between [^{18}F] FDG uptake in SNAP vs. AD.

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. SNAP: Suspected non-Alzheimer's pathophysiology; AD: Alzheimer's disease; 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 (within a range of 5mm) is indicated with the corresponding BA.

Analysis	Cluster level					Voxel-level		
	cluster p(FWE-corr)	cluster p(FDR-corr)	Cluster extent	Cortical Region	Z score of maximum	Talairach coordinates	Cortical region	BA
SNAP – AD (areas of reduced glucose metabolism in AD as compared to SNAP)	0,000	0,000	5077	R Temporal	3,61	48,-60,30	Superior temporal gyrus	39
				R Frontal	3,57	26,20,50	Middle Frontal gyrus	8
				R Parietal	3,48	36,-30,62	Postcentral gyrus	3

Table 3. Numerical results of SPM comparisons between [^{18}F] FDG uptake in AD and SNAP vs. CG.

Analysis	Cluster level					Voxel-level		
	cluster p(FWE-corr)	cluster p(FDR-corr)	Cluster extent	Cortical Region	Z score of maximum	Talairach coordinates	Cortical region	BA
CG – AD (areas of reduced glucose metabolism in AD as compared to CG)	0,000	0,000	25545	R parietal	6,98	44,-62,36	Angular gyrus	39
				R parietal	6,90	50,-48,38	Inferior parietal lobule	40
				R limbic	6,43	2,-40,34	R cingulate gyrus	31
	0,000	0,000	10762	R frontal	6,38	34,8,54	Middle frontal gyrus	6
				L frontal	5,67	-28,30,44	Middle frontal gyrus	8
				L frontal	5,54	-24,24,52	Superior frontal gyrus	8
CG – SNAP (areas of reduced glucose metabolism in SNAP as compared to CG)	0,011	0,008	3124	R limbic	4,31	6,-52,20	Posterior cingulate	23
				R parietal	3,90	-6,-64,24	Precuneus	31
				R limbic	3,70	4,-42,34	Cingulate gyrus	31

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. SNAP: Suspected non-Alzheimer's pathophysiology; AD: Alzheimer's disease; 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 (within a range of 5mm) is indicated with the corresponding BA.

Table 4: Region by region analysis of [^{18}F] FDG uptake in the cortex of SNAP subjects. Data reported are aimed to show the presence/absence of asymmetries of [^{18}F] FDG uptake in SNAP patients.

Cortical region	[^{18}F] FDG uptake* (mean±SD)	P value
Left frontal lobe	1,27±0,10	>0,05
Right frontal lobe	1,30±0,11	
Left limbic lobe	1,27±0,14	>0,05
Right limbic lobe	1,30±0,12	
Left occipital lobe	1,46±0,21	>0,05
Right occipital lobe	1,43±0,19	
Left parietal lobe	1,36±0,12	>0,05
Right parietal lobe	1,37±0,11	
Left temporal lobe	1,26±0,12	>0,05
Right temporal lobe	1,28±0,12	

* The values for [^{18}F] FDG uptake were obtained from the normalization of PET data using WFU pickatlas (see materials and methods section).

Figure 1: 3D rendering of data presented in Table 2 in (a) showing the results of SPM comparisons between [^{18}F] FDG uptake in SNAP vs. AD; significant hypometabolism in a wide cortical area that involves the right frontal, parietal and temporal lobes. In (b) the 3D rendering shows the overlap of the metabolic pattern observed in CG-SNAP (blue) and CG-AD (green) comparisons in the right limbic cortex. Coordinate and other regional details are presented in Table 2 and 3 respectively.

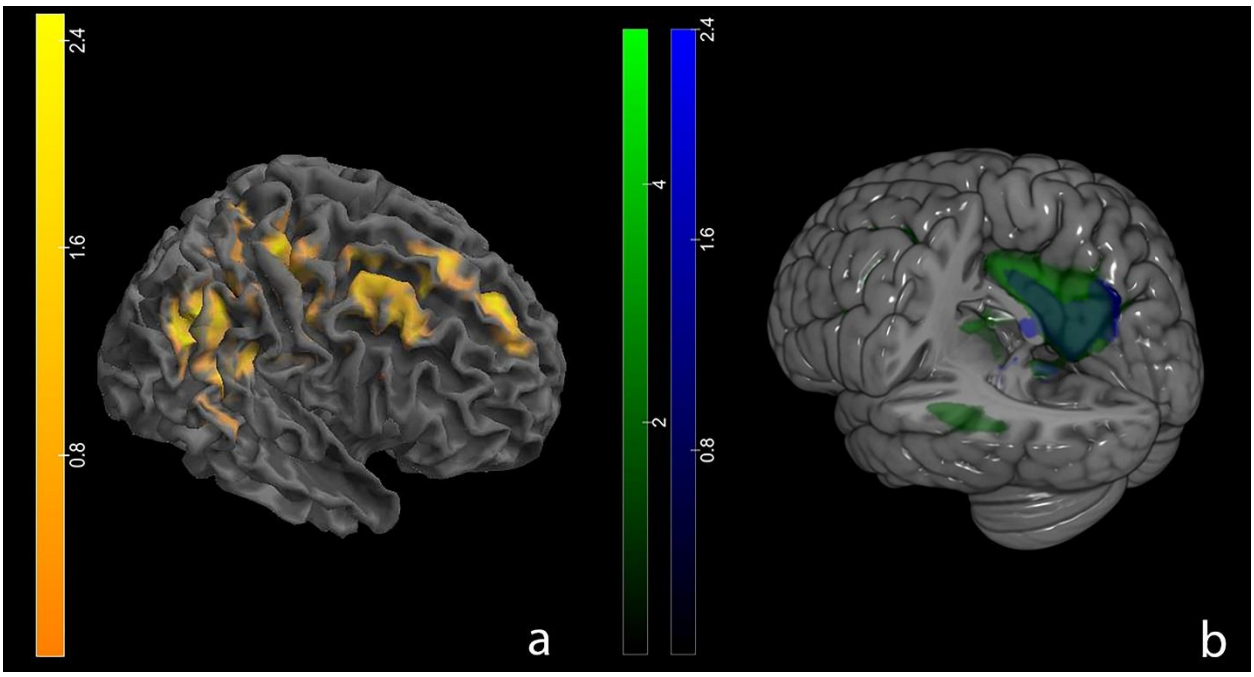


Figure 2: 3D rendering of data presented in Table 3 (CG-AD) showing the wide area of reduced glucose metabolism in AD as compared to CG(red). The reduction of [^{18}F] FDG uptake involved temporal, parietal, limbic and frontal cortex. (a) frontal view; (b) posterior view; view of the right hemisphere (c); view of the left hemisphere (d); bottom view (e) and upper view (f).

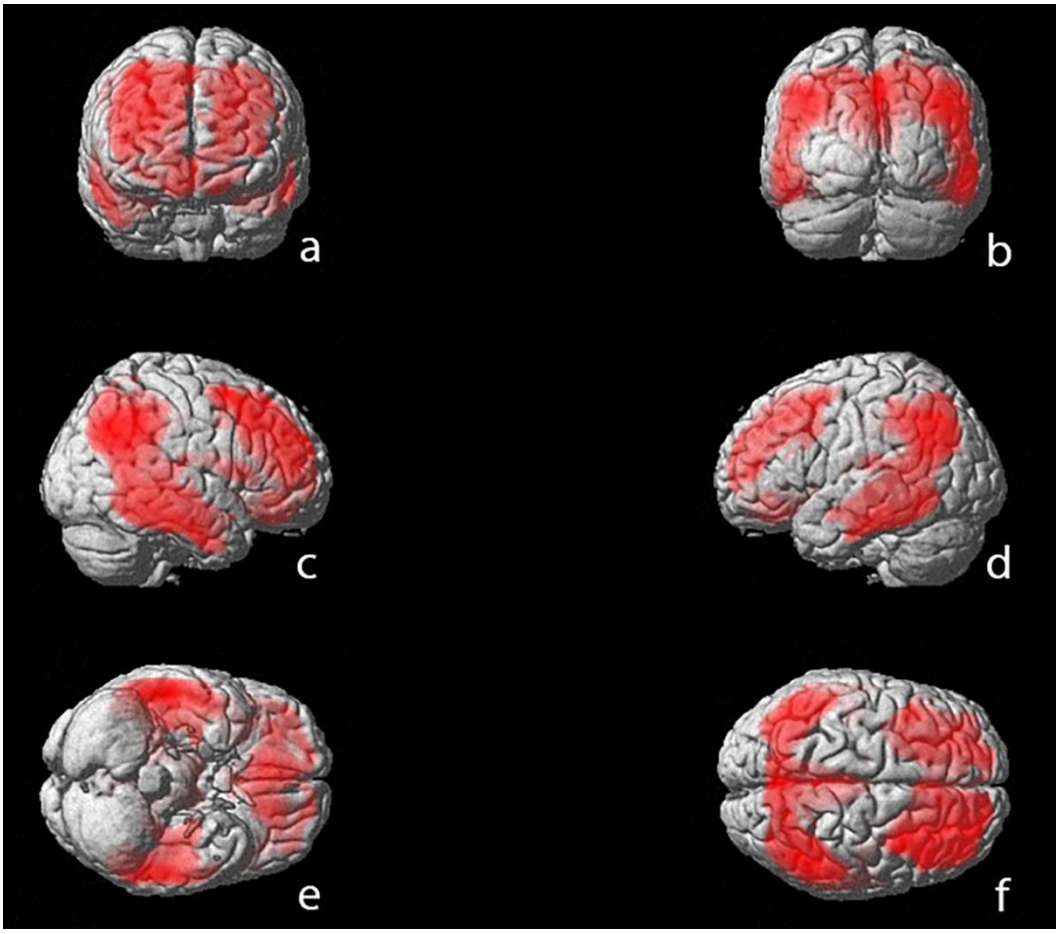
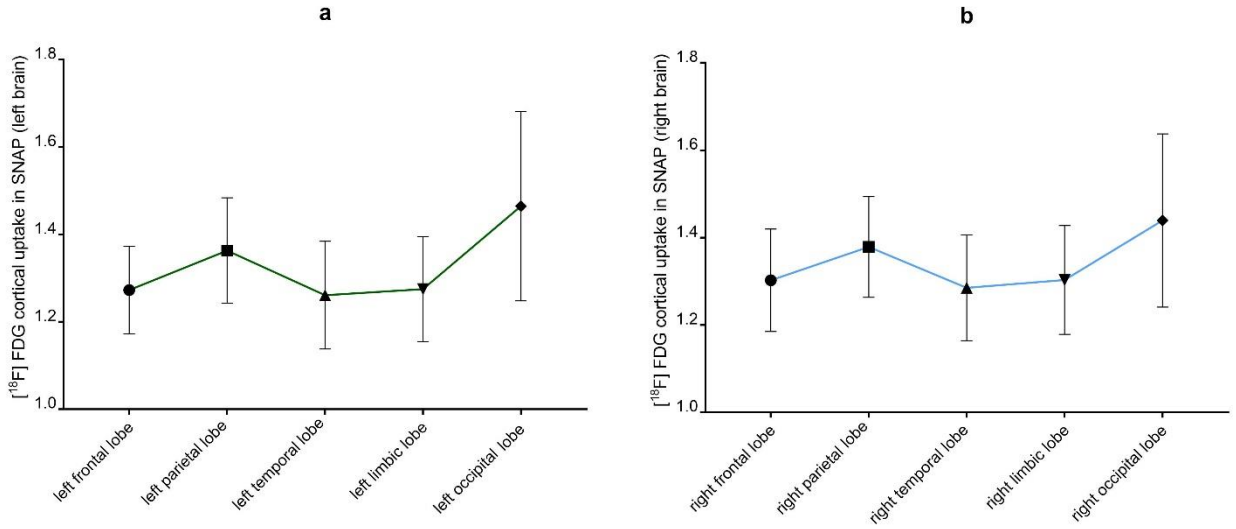


Figure 3. Graphical overview of data reported in Table 4. In figure (a) we report the values of [^{18}F] FDG uptake in the left hemisphere and in (b) the values for the right hemisphere. Despite [^{18}F] FDG uptake is lower in temporal and frontal lobe bilaterally than in the parietal lobes (with a pattern similar to that of frontotemporal dementia) the difference was not statistically significant.



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