



## Basal forebrain metabolism in Alzheimer's disease continuum: relationship with education



Brandt Nicolas<sup>a,\*</sup>, Dodich Alessandra<sup>b</sup>, Perani Daniela<sup>c,d</sup>, Ratib Osman<sup>a,e</sup>, Trombella Sara<sup>b,f</sup>, Frisoni Giovanni B<sup>f,g</sup>, Garibotto Valentina<sup>a,b,e,\*\*</sup>, for the Alzheimer's Disease Neuroimaging Initiative<sup>1</sup>

<sup>a</sup> Faculty of Medicine, Geneva University, Geneva, Switzerland

<sup>b</sup> NIMTlab, Neuroimaging and Innovative Molecular Tracers Laboratory, University of Geneva, Geneva, Switzerland

<sup>c</sup> Vita-Salute San Raffaele University and Division of Neuroscience San Raffaele Scientific Institute, Milan, Italy

<sup>d</sup> Nuclear Medicine Unit, San Raffaele Hospital, Milan, Italy

<sup>e</sup> Department of Radiology and Medical Informatics, Geneva University Hospitals and University of Geneva, Geneva, Switzerland

<sup>f</sup> Memory Center and LANVIE-Laboratory of Neuroimaging of Aging, Geneva University Hospitals, Geneva, Switzerland

<sup>g</sup> Laboratory of Alzheimer's Neuroimaging and Epidemiology, IRCCS Fatebenefratelli, Brescia, Italy

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### ABSTRACT

We analyzed education, as a proxy of cognitive reserve, and the cholinergic pathway in Alzheimer's disease (AD), to test the hypothesis that education might modulate the relationship between clinical symptoms and metabolic and structural changes in AD. We included 84 subjects and compared between diagnostic groups and different educational levels the glucose metabolism of basal forebrain (BFM) and volume of the basal forebrain, the major cholinergic structure, and hippocampus (HM) (and hippocampal volume), a relevant projection site for the basal forebrain. Correlations with the global cognitive status and education in the whole sample were also performed. Patients with AD dementia showed reduced basal forebrain volume, hippocampal volume, and HM compared with controls. In the whole group, the global cognitive status was positively correlated with BFM and HM. Among high-educated subjects, mild cognitive impairment showed higher BFM and HM in comparison to other diagnostic groups. Our results suggest that in mild cognitive impairment subjects with a higher educational level, cholinergic activity is up-regulated and this appears to have a compensatory effect, which may be lost in later symptomatic stages.

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### 1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia, present in 50%–75% of cases and mostly concerning individuals aged over 60 years (Prince et al., 2016). Before overt AD dementia, patients usually present a syndromic picture characterized by mild cognitive impairment (MCI), while functional autonomy is still

preserved. The relationship between pathology evolution and clinical symptoms severity is not linear. Disease progression might vary according to other risk factors (e.g. de Bruijn and Ikram, 2014) and modulatory variables, such as the cognitive reserve (CR). CR has been introduced to describe the different individual susceptibilities to the disease and it is associated with multiple life experiences, for example, education, occupational level, and leisure activities, which exert a significant compensatory effect on the clinical expression of neurodegenerative diseases (Stern et al., 2018). The hypothesis that CR, often approximated using the years of formal education (Stern et al., 2018; Valenzuela and Sachdev, 2006), modulates the clinical presentation and progression of neurodegenerative diseases obtained strong confirmation by imaging studies, showing that among AD patients with comparable disease severity, those with higher CR exhibit more severe brain dysfunction, already in the MCI phase (Arenaza-Urquijo and Vemuri, 2018; Garibotto et al., 2008; Morbelli and Nobili, 2014). Recent evidence suggests that the modulatory effect of education might also extend to the cholinergic system (Garibotto et al., 2013; Kim et al., 2012; Marcone et al.,

\* Corresponding author at: EPFL, 1015, Lausanne, Switzerland. Tel.: +41223727252; fax: +41223727169.

\*\* Corresponding author at: Division of Nuclear Medicine, Geneva University Hospitals Rue Gabrielle-Perret-Gentil 4, 1205 Geneva, Switzerland. Tel.: +41223727252; fax: +41223727169.

E-mail addresses: [Nicolas.brandt-dit-grieurin@epfl.ch](mailto:Nicolas.brandt-dit-grieurin@epfl.ch) (B. Nicolas), [valentina.garibotto@hcuge.ch](mailto:valentina.garibotto@hcuge.ch) (G. Valentina).

<sup>1</sup> The data analyzed in this work were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database ([adni.loni.usc.edu](http://adni.loni.usc.edu)). The ADNI investigators contributed to the design and implementation of ADNI and/or provided data but did not participate in these analyses and article. A complete listing of ADNI investigators can be found at: [http://adni.loni.usc.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Acknowledgement\\_List.pdf](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf).

2012), which is selectively affected early in AD (Grothe et al., 2014; Hall et al., 2008).

The basal forebrain (BF) represents one of the main cholinergic structures in the brain and includes the diagonal band of Broca, the substantia innominata, the medial septum nuclei, and the nucleus basalis of Meynert. According to Mesulam's nomenclature (Mesulam et al., 1983), as modified and implemented by Zaborszky (Zaborszky et al., 2008), the cholinergic nuclei can be classified in compartments Ch1 to Ch4: Ch1 and Ch2 mainly corresponding to the medial septum and the vertical limb of the diagonal band, Ch3 to the horizontal limb of the diagonal band, and Ch4 to the nucleus basalis of Meynert. BF atrophy has been extensively observed both in patients with MCI and patients with dementia (Grothe et al., 2012, 2014, 2016; Hall et al., 2008; Kilimann et al., 2014; Teipel et al., 2016), and it is associated to cognitive impairment and amyloid positivity (Grothe et al., 2010; Kerbler et al., 2015; Richter et al., 2014; Teipel et al., 2014). Notably, although the neocortex and amygdala are supplied by cholinergic neurons in the posterior basal nucleus of Meynert, BF sub-regions Ch1 and Ch2 represent the main origins of cholinergic projections to the hippocampus (HM), possibly stimulating processes of neuroplasticity and neurogenesis in this region (Colgin et al., 2003; Grothe et al., 2010). Because both BF and HM are well known areas of early neurodegeneration in AD (Jack et al., 2018; Kilimann et al., 2014; Mosconi et al., 2005; Sabri et al., 2018) and they have been both related to neuroplasticity processes, it is conceivable that these regions are those sensitive to modulatory phenomena such as CR. However, to the best of our knowledge, studies investigating this hypothesis are still sparse. A previous study comparing in healthy controls (HCs), patients with MCI, and patients with AD the uptake of [<sup>18</sup>F]-fluorodeoxyglucose (FDG) in the BF provided evidence of a higher FDG uptake in MCI compared with the other 2 groups (Kim et al., 2012). Moreover, although in low-educated subjects the relationship between MMSE and FDG uptake was mainly represented by a positive linear regression curve, those with higher education showed an inverted-U relationship (Kim et al., 2012). A previous study of our group using the positron emission tomography (PET) tracer carbon 11-labeled N-methyl-4-piperidyl-acetate to quantify acetylcholinesterase activity, reported in prodromal and early AD phase a significant positive association between the activity of the cholinergic projections in the left and right HMs and CR measurements (Garibotto et al., 2013). These findings suggest that the CR mechanisms might result in an increased or preserved function of the cholinergic system in these subjects. Although volumetric changes in the HM represent a validated imaging biomarker in AD (Jack et al., 2018) and BF atrophy has been extensively reported in AD and MCI subjects (Kilimann et al., 2014; Teipel et al., 2016), metabolic changes in these regions as evaluated by FDG-PET often

went unnoticed possibly because of low spatial resolution of early generation PET scans or to possible inaccuracies in automated voxel-based analyses when performing statistical comparison at whole-brain level. Although spatial resolution of conventional PET scanners for small or deep subcortical structures, such as the BF, is still limited (Heiss et al., 2004), the introduction of the high-resolution research tomograph (HRRT) allows a high spatial resolution (approximately 2.5 mm) and a more reliable measurement of FDG uptake in these structures. Thus, in this study, by using volumetric MRI and HRRT-PET data, we aim to (1) examine in different AD-related clinical stages the significant alterations in volume and glucose metabolism in the BF and HM and (2) assess the association of these metabolic and volumetric indexes with the educational level.

## 2. Material and methods

### 2.1. Subjects

Cohort data for this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.ucla.edu>), a large longitudinal multicenter initiative with the purpose of studying and identifying helpful biomarkers for early detection and staging of AD. This database encompasses clinical, genetic, imaging, and biochemical items. Such biomarkers are also useful for differential diagnosis from other causes of dementia and serve as surrogate markers for treatment efficacy. ADNI is composed of 4 phases: ADNI 1, ADNI GO, ADNI 2, and ADNI 3. Each new phase increases the amount of patients and study techniques included in the study.

The participants of the present study were selected from the ADNI 1, ADNI 2, and ADNI GO databases if they had undergone HRRT-PET and a volumetric MRI. Diagnostic categories are detailed in Table 1. Subjects without information on disease severity (i.e., MCI belonging to ADNI 1 initiative) were excluded. Besides, among patient subgroups, only those with an amyloid-positive profile were considered. A final sample of 84 subjects was included in the present study, including 28 HCs and 56 amyloid-positive subjects with cognitive deficits or complaints. See Table 2 for demographic and clinical features.

### 2.2. Measure of education

To analyze metabolic and volume differences in subjects with high and low education, the whole sample was divided in 2 groups based on the median of years of education (Morbelli et al., 2013). Subjects with 16 or more years of education were assigned to the high-education group, and those with less than 16 years to the low-education group.

**Table 1**

Table defining the diagnostic criteria for each disease stage

CN	Normal aging/cognitively normal	ADNI 1/GO/2	CN participants are the control subjects in the ADNI study. They show no signs of depression, mild cognitive impairment, or dementia.
SMC	Significant memory concern	ADNI 2	SMC participants score within normal range for cognition (or CDR = 0) but indicate that they have a concern and exhibit slight forgetfulness. The informant does not equate this as progressive memory impairment nor considers this as consistent forgetfulness.
EMCI	Early mild cognitive impairment	ADNI GO/2	MCI participants have reported a subjective memory concern either autonomously or via an informant or clinician. However, there are no significant levels of impairment in other cognitive domains, essentially preserved activities of daily living and there are no signs of dementia. Levels of MCI (early or late) are determined using the Wechsler Memory Scale Logical Memory II.
LMCI	Late mild cognitive impairment	ADNI GO/2	
AD	Alzheimer's disease	ADNI 1/GO/2	AD participants have been evaluated and meet the NINCDS/ADRDA criteria for probable AD.

Key: ADNI, Alzheimer's Disease Neuroimaging Initiative; ADRCA, Alzheimer's Disease and Related Disorders Association; CDR, Clinical Dementia Rating; MCI, mild cognitive impairment; NINCDS, National Institute of Neurological and Communicative Disorders and Stroke.

<http://adni.loni.usc.edu/study-design/background-rationale/>.

**Table 2**  
Demographic and clinical characteristics of the diagnostic groups

Participant characteristics	HC (n = 28)	SMC (n = 7)	MCI (n = 33) (19 EMCI, 14 LMCI)	AD (n = 16)	Statistics
Age in years (M ± SD)	73.9 ± 7.1	70.8 ± 6.6	73.3 ± 7.4	75.6 ± 8.0	<i>p</i> = 0.52
Gender (F/M)	10/18	5/2	13/20	4/12	<i>p</i> = 0.208
Education, year (M ± SD)	16.7 ± 2.8	14.9 ± 3.2	15.5 ± 2.3	16.2 ± 2.1	<i>p</i> = 0.18
MMSE (M ± SD) <sup>a</sup>	28.7 ± 1.5 <sup>c</sup>	28.6 ± 2.1 <sup>c</sup>	27.7 ± 1.5 <sup>b,c</sup>	23.3 ± 2.5	<i>p</i> < 0.0001
APOE4 (0,1,2)	23/4/1	2/4/0, 1 n.a.	16/13/3, 1 n.a.	7/8/1	-
Amyloid (+,-)	14/12, 2 n.a.	7/0	33/0	16/0	-

Key: HC, healthy control; SMC, significant memory complaint; MCI, mild cognitive impairment (EMCI = early, LMCI = late); AD, Alzheimer's disease; M ± SD: mean ± standard deviation; FDG, fluorodeoxyglucose; PET, positron emission tomography.

<sup>a</sup> MMSE score obtained at the clinical visit closest to the FDG-PET examination.

<sup>b</sup> Significantly different compared with HC *p* = 0.024.

<sup>c</sup> Significantly different compared with AD *p* < 0.0001.

### 2.3. Amyloid status

One of the ADNI PET core laboratories (Jagust Lab, UC Berkeley, 17) made amyloid status for patients enrolled in the ADNI GO and ADNI 2 studies available. The amyloid status for ADNI 1 patients was included in a later phase if they underwent AV45. Amyloid positivity was established for a cortex-to-whole cerebellum standardized uptake value ratio (SUVR) higher than 1.11. First, cortical gray matter regions of interest (ROIs) (frontal, anterior/posterior cingulate, lateral parietal, and lateral temporal) and reference regions were defined on the native-space MRI scans using FreeSurfer (version 4.5.0) for segmentation and parcellation. The corresponding AV45 scan was then coregistered to the MRI scan, and mean tracer uptake was calculated within cortical and reference regions. Finally, cortex-to-whole cerebellum SUVR was derived for each subject by dividing the average cortical AV45 uptake to the average cerebellar uptake. The procedure is further detailed online. Images for which SUVR values were not available (11C-PIB-PET [11C-labeled Pittsburgh Compound-B-positron emission tomography]) or with borderline values were evaluated by local expert nuclear physicians according to validated visual assessment following European Medicines Agency/US Food and Drug Administration-approved standard operating procedures.

### 2.4. Data acquisition

For all the 3 phases of the ADNI study, both FDG-PET and T1-weighted MRI images were acquired at screening or baseline time points. For a few patients who were present at different baseline/screening images, only those in the earliest phases were considered. Images were processed using statistical parametric mapping (SPM8, Wellcome Trust Center for Neuroimaging) software tool. Preprocessed PET and raw T1 MRI images were first manually reoriented, with the origin set at the intersection between the anterior commissure and the interhemispheric plane.

### 2.5. MRI data acquisition and preprocessing

We considered the raw 3D T1 MRI series acquired at the closest date to the FDG-PET examination. ADNI GO/2 MRI was acquired on 3T MRI scanners, and ADNI 1 MRI was acquired on either 1.5 T or 3T scanners using scanner-specific T1-weighted sequences. A more detailed description of the imaging protocols is reported online. MRI images were first automatically segmented into gray matter (C1), white matter (C2), and CSF (C3) using the SPM8 segmentation routine. C1 and C2 were then registered to their average template using the DARTEL toolbox (Ashburner, 2007). The resulting individual flow fields were used to warp the gray matter segments. Voxel value was modulated to preserve the total amount of gray matter present before warping. MRI images were finally smoothed using a Gaussian kernel of 4 mm.

### 2.6. FDG-PET data acquisition and preprocessing

HRRT-PET scans were acquired on a Siemens ECAT tomograph. For the present study, PET series already available in preprocessed form were selected, that is, 6 five-minute frames (ADNI 1) or 4 five-minute frames (ADNI GO/2) scans. Images were coregistered, averaged, and reoriented into a standard voxel image grid (more details online). The reoriented PET images were first coregistered to their corresponding MRI image. PET images were then warped using the corresponding MRI scan flow field obtained from the DARTEL registration. PET images were modulated to preserve total amount of activity and finally smoothed using a Gaussian kernel of 4 mm. See Fig. 1 for the image preprocessing steps.

### 2.7. Region of interest analysis

Values from specific ROIs were extracted using the SPM anatomy toolbox, which allows for integration of functional imaging data and probabilistic cytoarchitectonic maps (Eickhoff et al., 2005).

For the classification of the cholinergic nuclei in the BF, we followed Mesulam's nomenclature, as modified and implemented by Zaborszky (Zaborszky et al., 2008), defining Ch1 to Ch4 compartments. Ch1 and Ch2 mainly correspond to the medial septum and the vertical limb of the diagonal band, Ch3 to the horizontal limb of the diagonal band, and Ch4 to the nucleus basalis of Meynert (Zaborszky et al., 2008). Thus, the chosen ROIs were defined as follows: for the BF, left and right medial septal nucleus and diagonal band of Broca (Ch1-3L and Ch1-3R) and left and right nucleus basalis of Meynert (Ch4L and Ch4R). For the HM, we considered bilateral dentate gyrus, CA1, CA2, CA3, subiculum, entorhinal cortex, and hippocampal-amygdaloid transitional area regions (see Fig. 2 for graphical representation of BF and hippocampal ROIs). We used the "all assigned" options for voxel assignment. Mean metabolic values of the BF and HM subregions were calculated on the coregistered, warped, modulated, and smoothed HRRT-PET images. Values were normalized by mean vermis activity. For the metabolic value of the whole BF (BFM) and HM, a mean weighted by subregional volume was calculated. In the analyses of gray matter volume, mean voxel values were extracted from the warped, modulated, and smoothed gray matter images. Regional volume was determined by multiplying the mean voxel values with the total regional volume of the probabilistic map and subsequently correcting for total intracranial volume, which was calculated as the sum of gray matter, white matter, and CSF volumes (BF volume: BFV; hippocampal volume: HV).

### 2.8. Statistical analysis

Statistical analyses were performed on whole BF and whole HM values (BFM, HM, BFV, and HV) as well as on BF subregions. To compare the different diagnostic groups, we performed one-way

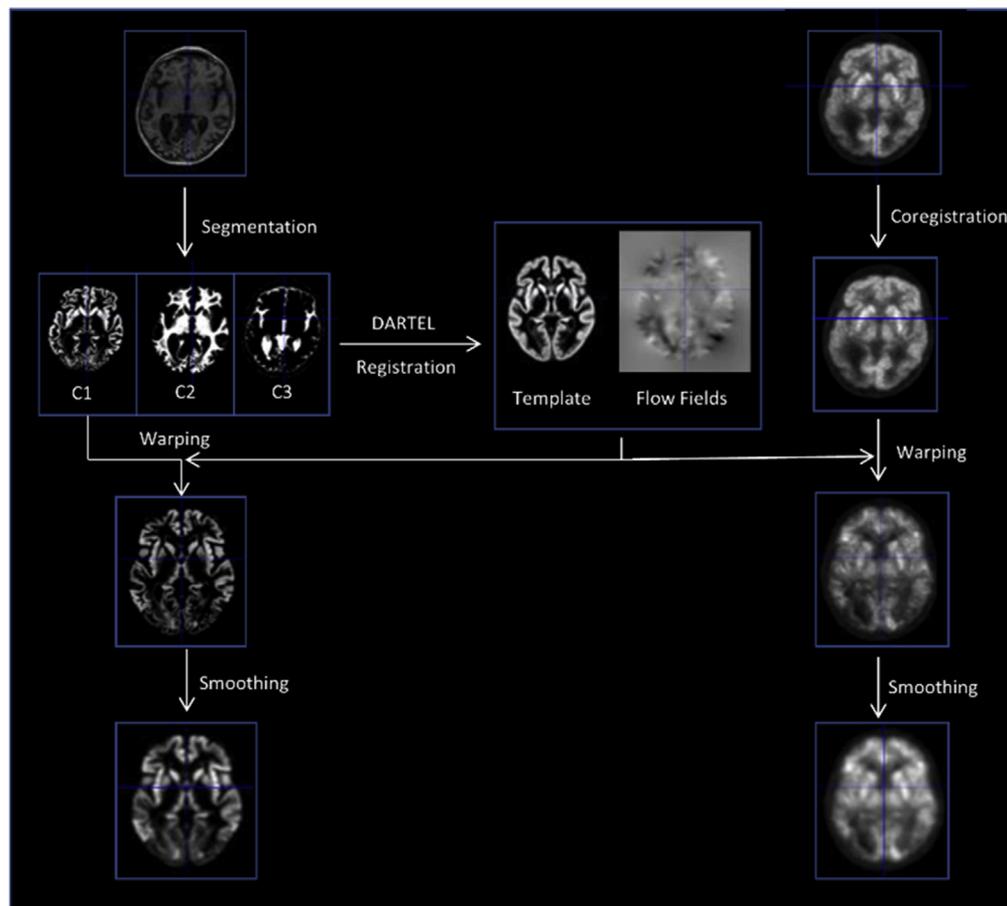


Fig. 1. Flowchart of the image processing procedure.

ANOVA (Least significant difference post hoc test,  $p < 0.05$ ) on glucose metabolism (BF: BFM and subregions; HM) and gray matter volume (BF: BFV and subregions; HV) using age, gender, and ApoE4 status as nuisance variables.

To investigate the association with education, we computed partial correlations (Pearson's correlation,  $p < 0.05$ ) in the whole sample between this variable and metabolic/volume values (BFM, HM, BFV, and HV). Age, gender, MMSE, and ApoE4 status were selected as nuisance variables. To further test the hypothesis driving the concept of CR (i.e., in subjects with high education, cognitive impairment is less severe than expected for the amount of pathological damage), we also performed a correlation analysis between education and the residuals derived from a regression analysis of MMSE on metabolic/volume variables, controlling for the nuisance variables (age, gender, and APOE). Finally, to further test this association, an exploratory whole-brain voxel-by-voxel analysis was performed in the whole sample (SPM8, one-sample  $t$ -test, proportional scaling to global value, family-wise error-corrected  $p$ -value  $< 0.05$ ) evaluating possible correlations (positive and negative) between years of education and structural/functional measurements.

One-way ANOVA was then performed in high-education and low-education subgroups separately, to compare metabolic and gray matter differences among BF and hippocampal regions as well as on BF subregions. Significant memory complaint subjects were excluded because of the small sample size ( $n = 7$ , 2 low-educated and 5 high-educated subjects).

To specifically investigate the mechanisms at the base of cognitive resilience (i.e., maintaining high cognitive performances despite significant AD pathology), we focused on cognitively

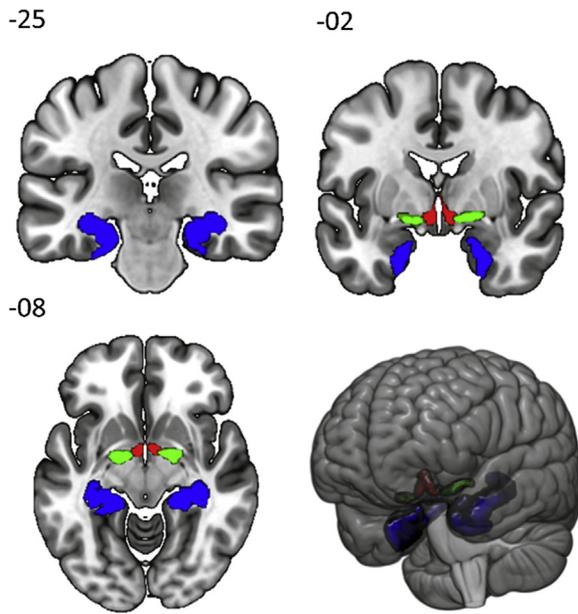
impaired subjects, performing a  $2 \times 2$  ANOVA to investigate the interaction between education (factor 1: high vs low) and clinical stage (factor 2: MCI vs AD) in functional (BF: BFM and subregions; HM) and structural variables (BF: BFV and subregions; HV). In all the analyses focused on education, age, gender, ApoE4 status, and MMSE score were entered as nuisance variables.

Finally, to investigate the relationship between cognitive impairment and structural/functional changes, we analyzed in the whole sample the partial correlation (Pearson's correlation,  $p < 0.05$ ) between MMSE scores and imaging variables for BF (BFM, BFV) and the hippocampus (HM, HV). These analyses were performed in the overall group and in high- and low-educated subgroups, separately. Covariates were age, gender, and ApoE4 status, as well as years of education. Statistical analyses were performed using SPSS v23 statistical software (SPSS, Inc, Chicago, IL).

### 3. Results

#### 3.1. Functional and structural differences between diagnostic groups

First, we compared metabolic and volume differences in BF and hippocampal regions across all diagnostic groups. BFM was not significantly different across groups ( $F(3,75) = 1.85, p = 0.15$ ) (Table 3); however, the analyses of BF subregions showed significant differences in the left Ch4 ( $F(3,75) = 2.85, p = 0.043$ ), with MCI groups showing higher metabolic values in comparison to AD ( $p = 0.016$ ) and HC ( $p = 0.048$ ) (Table 4). The comparison showed that HM was significantly lower in AD than in the other groups ( $F(3,75) = 3.01,$



**Fig. 2.** Cytoarchitectonic maps of the basal forebrain (Ch1-3 [red] and Ch4 [green]) and hippocampus [blue]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

$p = 0.036$ ). Patients with AD showed reduced BFV ( $F(3,75) = 3.12$ ,  $p = 0.031$ ) and HV ( $F(3,75) = 5.29$ ,  $p = 0.002$ ) in comparison to all other subgroups (see Table 3 for post hoc). The analyses on BF subregions confirmed a reduced volume in AD compared with all the other groups, particularly in Ch4 ( $F(3,75) = 3.28$ ,  $p = 0.026$ ).

### 3.2. Effect of education on brain metabolism and volume and correlation with cognitive status

In a second step, we investigated the relationship between education and structural/functional parameters. The results showed in the whole sample a negative correlation between education and BFV ( $r = -0.24$ ,  $p = 0.036$ ), in agreement with previous observations showing an inverse association between CR and pathological burden, when taking into consideration disease severity (Arenaza-Urquijo et al., 2011; Borroni et al., 2009; Kemppainen et al., 2008; Sole-Padullés et al., 2009). However, no significant association was found between education and the residuals derived from a regression analysis of MMSE on BFV ( $\rho = 0.16$ ,  $p = 0.15$ ). The exploratory whole-brain voxel-by-voxel analysis performed in the whole sample showed no significant results.

**Table 3**  
Comparison between metabolic and gray matter values extracted from ROIs in all subgroups

Regions of interest	HC n = 28	SMC n = 7	All MCI n = 33	AD n = 16	ANOVA
BFM	0.73 ± 0.09	0.74 ± 0.04	0.76 ± 0.08	0.71 ± 0.08	$p = 0.15$
HM	0.89 ± 0.13	0.96 ± 0.09 <sup>a</sup>	0.91 ± 0.13 <sup>a</sup>	0.79 ± 0.14	$p = 0.036$
BFV	454 ± 52 <sup>b</sup>	480 ± 56 <sup>a</sup>	452 ± 58 <sup>b</sup>	405 ± 40	$p = 0.031$
HV	6696 ± 860 <sup>c</sup>	6997 ± 838 <sup>a</sup>	6568 ± 1052 <sup>b</sup>	6103 ± 993	$p = 0.002$

Key: AD, Alzheimer's disease; HC, healthy control; ROIs, regions of interest; SMC, significant memory complaint; MCI, mild cognitive impairment; BFM, mean BF metabolism; HM, hippocampal metabolism; BFV, BF volume; HV, hippocampal volume.

ANOVA  $p$ -values below 0.05 are highlighted in bold.

<sup>a</sup>  $p < 0.05$  compared with AD.

<sup>b</sup>  $p < 0.01$  compared with AD.

<sup>c</sup>  $p < 0.001$  compared with AD.

**Table 4**  
Comparison between metabolic values of BF subregions in all subgroups

Regions of interest	HC n = 28	SMC n = 7	MCI n = 33	AD n = 16	ANOVA
Ch 1-3 left	0.64 ± 0.09	0.65 ± 0.06	0.67 ± 0.07	0.66 ± 0.09	$p = 0.46$
Ch 1-3 right	0.66 ± 0.08	0.68 ± 0.05	0.69 ± 0.08	0.67 ± 0.09	$p = 0.37$
Ch 4 left	0.73 ± 0.10 <sup>a</sup>	0.77 ± 0.04	0.77 ± 0.10	0.69 ± 0.09 <sup>a</sup>	$p = 0.043$
Ch 4 right	0.79 ± 0.10	0.77 ± 0.07	0.82 ± 0.10	0.76 ± 0.09	$p = 0.22$

Key: AD, Alzheimer's disease; HC, healthy control; SMC, significant memory complaint; MCI, mild cognitive impairment; BF, basal forebrain.

ANOVA  $p$ -values below 0.05 are highlighted in bold.

<sup>a</sup>  $p < 0.05$  compared with MCI.

The effect of education has been then evaluated dividing subjects in low-/high-education subgroups according to the median years of education (16 years). First, we performed a 2 (high vs low education) × 3 (HC vs MCI vs AD) ANOVA on age. The analysis showed no significant effect on age of educational level ( $F(1,71) = 0.53$ ,  $p = 0.47$ ), diagnostic groups ( $F(2,71) = 0.056$ ,  $p = 0.95$ ), or interactions across these factors ( $F(2,71) = 2.7$ ,  $p = 0.07$ ). Moreover, no differences were found between high- and low-education subgroups in APOE positivity ( $X^2(1) = 2.5$ ,  $p = 0.11$ ) or gender ( $X^2(1) = 1.3$ ,  $p = 0.2$ ). No significant differences were found in age and gender between diagnostic groups considering low-educated (age:  $F(2,22) = 1.21$ ,  $p = 0.32$ ; gender: Fisher's exact test: 0.31  $p = 1.00$ ) and high-educated subjects (age:  $F(2,49) = 2.24$ ,  $p = 0.12$ ; gender: Fisher's exact test: 0.79  $p = 0.68$ ). In low-educated subjects, we found a trend to significance in APOE e4 positivity (Fisher's exact test: 6.35,  $p = 0.05$ ), whereas no significant differences were found in highly educated subjects (Fisher's exact test: 3.4,  $p = 0.19$ ).

In the high-education subgroup, patients with MCI showed higher metabolic values compared with HCs in BFM ( $F(2,44) = 4.18$ ,  $p = 0.022$ , least significant difference post hoc test  $p = 0.006$ ), Ch1-3L ( $F(2,44) = 5.16$ ,  $p = 0.010$ ), and Ch4LF ( $F(2,44) = 4.89$ ,  $p = 0.012$ ) (see Fig. 3), which was associated to a possible trend toward significance in Ch4L gray matter volume ( $F(2,44) = 3.07$ ,  $p = 0.056$ ). Moreover, high-educated MCI subjects showed higher hippocampal metabolism in comparison to HC, although not reaching significance ( $F(2,44) = 3.14$ ,  $p = 0.05$ ). No significant differences were present between the low-education subgroups.

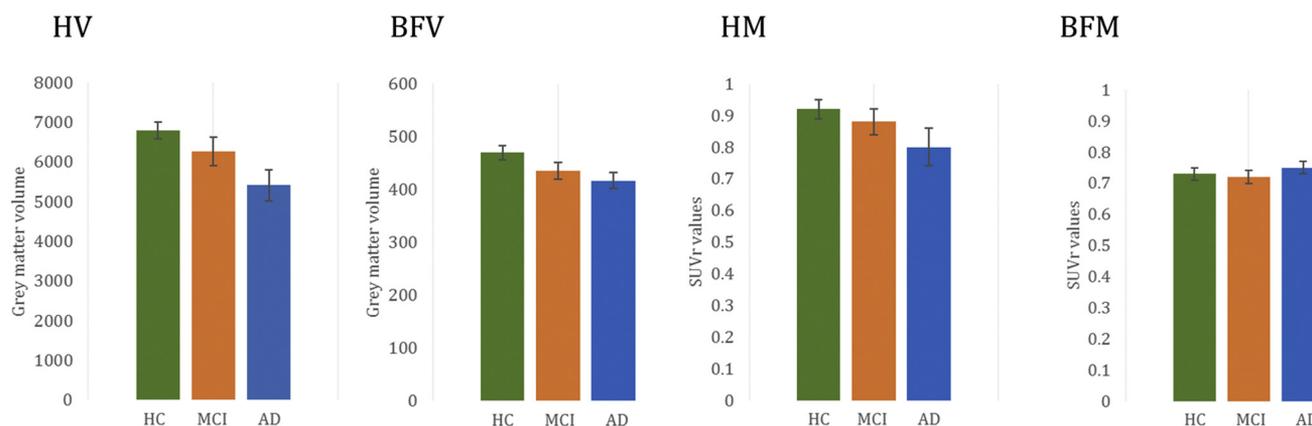
The 2 × 2 ANOVA comparing metabolic and structural values between MCI and AD divided accordingly to high/low education showed a significant interaction between group and education in BFM ( $F(1,40) = 4.29$ ,  $p = 0.045$ ) (see Fig. 3). These results were confirmed in Ch1-3L ( $F(1,40) = 4.69$ ,  $p = 0.036$ ), Ch1-3R ( $F(1,40) = 6.62$ ,  $p = 0.014$ ), and Ch4R ( $F(1,40) = 4.27$ ,  $p = 0.045$ ). No significant interactions were found in hippocampal variables.

The correlation analyses between the cognitive status (i.e., MMSE score) and metabolic/volume variables in the whole group showed that MMSE score was positively correlated with BFM ( $r = 0.226$ ,  $p = 0.046$ ) and HM ( $r = 0.376$ ,  $p = 0.001$ ). Besides, MMSE score significantly correlated to HV ( $r = 0.326$ ,  $p = 0.004$ ). These results were confirmed only in the high-educated group (BFM:  $r = 0.37$ ,  $p = 0.007$ ; HM:  $r = 0.45$ ,  $p = 0.001$ ; HV:  $r = 0.37$ ,  $p = 0.006$ ), whereas no significant results emerged in low-educated subjects.

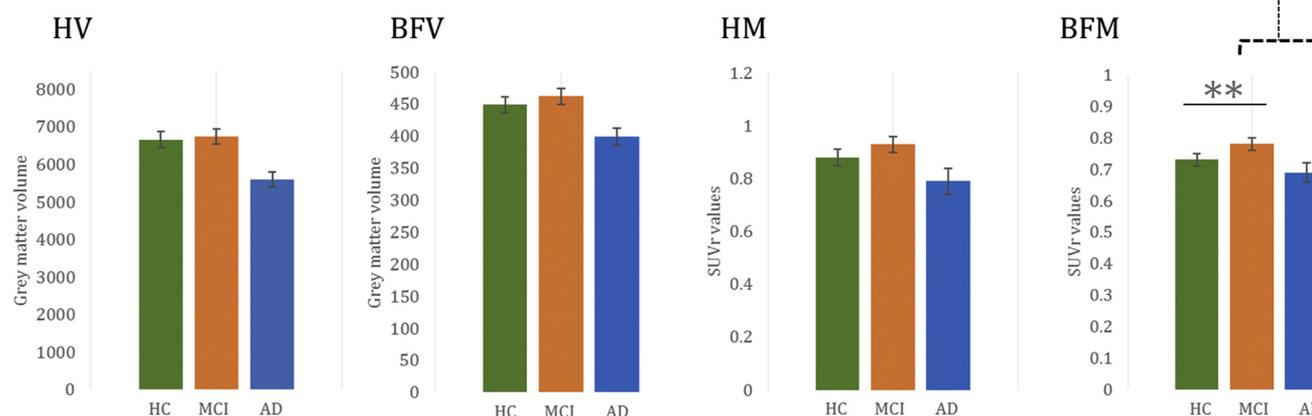
## 4. Discussion

In this study, we showed that education modulates the relationship between the clinical symptoms and the structural and functional integrity of 2 regions significantly affected by AD neurodegenerative processes (i.e., BF and hippocampus). Notably, we provided evidence of a significant interaction between education and clinical stage, suggesting a differential effect of this modulatory variable according to the clinical severity.

## Low-educated groups



## High-educated groups



**Fig. 3.** Bar charts representing the mean values of gray matter volume (HV, BFV) and metabolism (HM, BFM) extracted from the BF and hippocampus. Dotted lines represent the significant interaction in BFM between groups (MCI in orange, AD in blue), and education (low, high) is shown. Error bars represent standard errors. \* $p < 0.05$ , \*\* $p < 0.01$ . Abbreviations: HV, hippocampal volume, BFV, basal forebrain volume; HM, hippocampal metabolism; BFM, basal forebrain metabolism. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The current lack of effective treatment for AD emphasizes the importance of investigating CR, as it could play a significant role in delaying or slowing down the onset and progression of disease (Barulli and Stern, 2013). Of note, when analyzing separately subjects with higher and lower educational level, we observed only in the high-educated ones an increase in BF metabolic activity that was present specifically in the early symptomatic stage, namely in patients with MCI, compared with HC and patients with AD. These findings might suggest an underlying upregulation in cholinergic activity in the early symptomatic phases, particularly in subjects with high education, possibly representing a compensatory capacity that might be lost with disease progression. FDG-PET (Ashraf et al., 2015), fMRI studies (Clement and Belleville, 2010; Gigi et al., 2010), as well as studies investigating the cholinergic neurotransmission (Garibotto et al., 2013) in MCI and HC support this hypothesis, which is further reinforced by the significant results of the interaction analysis between educational level and clinical stage. To the best of our knowledge, only a single study, and in a limited patient population, addressed this issue studying BF with HRRT-PET, observing, in agreement with our data, a positive correlation between BF metabolism and MMSE, together with a metabolic

increase in MCI (Kim et al., 2012). The increase of BF metabolism in MCI subjects and the association with MMSE performances might be thus interpreted as possible compensative mechanisms for AD-induced dysfunction of structures targeted by the cholinergic innervation. Importantly, the presence of cholinergic plasticity has been previously reported, with the observation of a compensatory response maintaining or upregulating choline acetyltransferase activity namely in patients with MCI, and an association with CR has been postulated, even if not specifically investigated (DeKosky et al., 2002; Ikonovic et al., 2003; Mufson et al., 2015). Despite the mild effectiveness of acetylcholinesterase inhibitors reported in patients with AD (Wattmo et al., 2011), a recent functional magnetic resonance study (Richter et al., 2018) showed that acute rivastigmine treatment in MCI induced significant functional brain changes during an episodic memory task in the same brain regions recruited by HCs, suggesting a trend toward a “normalization” of neocortical activity.

The relationship between cholinergic system integrity and cognitive status is illustrated here by the positive correlation between BF metabolism and MMSE score, the latter also positively associated to hippocampal metabolism and volume. Hippocampal

atrophy and hypometabolism as measured by FDG-PET represent well-known biomarkers of AD neurodegeneration (Jack et al., 2018), and as expected, our data confirmed in patients with AD a significant functional and structural hippocampal damage. Notably, high-educated MCI subjects showed higher metabolism in this region compared with HCs.

Hippocampal hyperactivity has been already reported in subjects without dementia; however, this has been interpreted as an indicator of forthcoming hippocampal failure, as subjects with more rapid cognitive decline presented the greatest hippocampal activation in a fMRI task at T0 and the highest loss of activation in the same region at two-year follow-up (O'Brien et al., 2010). In our sample, this hyperactivation has been found specifically in high-educated subjects. Overall, these results are in line with the prevalent model of CR, suggesting that higher educated subjects might put in place additional compensatory mechanisms, despite a steeper clinical decline because of the accumulation of more pathological changes (Meng and D'Arcy, 2012).

It is important to underline that we measured volume and metabolism of the BF, which is usually studied for its cholinergic properties, but it is not an exclusively cholinergic structure. Indeed, the BF cortical projections include a relevant proportion of GABAergic neurons in the areas examined, which could contribute to the effects we observed, either directly or through a cholinergic modulation (Rosato-Siri et al., 2006; Sarter and Bruno, 2002; Yang et al., 2014). It should be noted, however, that the GABAergic population is less affected by the neurodegenerative process in AD, as compared with the cholinergic component (Palmer, 1996; Rossor et al., 1982). In addition, the relationship between GABAergic vulnerability and AD pathology remains unclear, as GABAergic dysfunction could involve both compensatory and direct pathological responses (see Govindpani et al., 2017).

In conclusion, this study provides evidence for a modulatory effect of education on the relationship between clinical presentation and functional and structural integrity in the BF and hippocampus. To achieve a sufficiently high spatial resolution, we included ADNI subjects who underwent an HRRT-PET scan. Despite that automated voxel-based analysis may fail to highlight hippocampal metabolic dysfunction (Mosconi et al., 2005), the implementation of high-resolution scan, as well as an ROI-based approach, allowed a more accurate hippocampal and BF FDG-PET quantification. A limitation of FDG-PET imaging interpretation is that it is only an indirect measuring of cholinergic activity compared with measures obtained with acetylcholinesterase markers such as [<sup>11</sup>C]-MP4A. Even using HRRT-PET, the small size of the BF, may also cause measurement errors and difficulties in clearly distinguishing its subregions. A strength of this study is the inclusion of amyloid-positive patients only. The homogeneity and the clear definition of patient sample is a crucial point, considering the significant correlation between neocortical amyloid accumulation and BF degeneration (Kerbler et al., 2015). Our study has also limitations worth discussing. The main one is the relative small sample size in each subgroup. Another is that we only considered cross-sectional data: longitudinal data would be required to show the temporal evolution of the differences we observed across diagnostic stages. A third limitation is that we observed the effect of education when adopting a median split approach, as in previous literature (Morbelli et al., 2013), but we could not show a continuous effect of education on BFM. This mismatch between the results in group and regression analyses might be possibly linked to the nonlinear relation between metabolism and education or to the small sample size and high educational level of our sample, common to other studies from the ADNI population (Mainta et al., 2018).

## Disclosure

The authors declare that they have no conflict of interests. This article does not contain any studies with human participants performed by any of the authors.

## CRediT authorship contribution statement

**Brandt Nicolas:** Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Dodich Alessandra:** Investigation, Formal analysis, Writing - review & editing. **Perani Daniela:** Writing - review & editing. **Ratib Osman:** Software, Writing - review & editing. **Trombella Sara:** Investigation, Formal analysis. **Frisoni Giovanni B:** Conceptualization, Writing - review & editing. **Garibotto Valentina:** Investigation, Formal analysis, Conceptualization, Methodology, Supervision, Resources, Writing - review & editing.

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