Atrophy, hypometabolism and clinical trajectories in amyloid negative probable AD patients

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Abstract

About 15% of patients clinically diagnosed with Alzheimer’s disease do not show high tracer retention on amyloid PET. The present study investigates the clinical and demographic features, patterns of brain atrophy and hypometabolism and longitudinal clinical trajectories of these patients, with a particular emphasis on the AD-mimic typical amnestic subgroup. 38 amyloid PET-negative patients carrying a pre-PET diagnosis of AD (Aβneg-AD) from 4 centers (Amsterdam, Melbourne, San Francisco and Caen) were included in this study (11/27 females/males; mean age=67±9). Detailed clinical histories, including the clinical diagnoses before and after the PET scan and at follow-up (>2 years), were collected. Patients were classified according to their pre-PET clinical phenotype as amnestic (memory predominant), non-amnestic (predominant language, visuospatial or frontal symptoms), or non-specific (diffuse cognitive deficits). Demographic, clinical, neuropsychological, MRI and FDG-PET data obtained at the time of the amyloid-PET scan were compared between Aβneg-AD subgroups, 27 Aβ-positive AD cases with a typical amnestic clinical presentation (Aβpos-AD; 14/13 females/males; mean age=71±10) and 29 Aβ-negative cognitively healthy controls (Aβneg-HC; 15/14 females/males; mean age=69±12) matched for age, gender and education. There were 19 amnestic, 13 non-amnestic, and 7 non-specific Aβneg cases. Aβneg-AD subgroups did not differ in age, gender, education or APOE4 proportion. After the PET scan, clinicians altered the diagnosis in 68% of Aβneg-AD cases including 44% of amnestic versus 94% of non-amnestic and non-specific cases. Amnestic Aβneg-AD were most often reclassified as frontotemporal dementia, non-amnestic as frontotemporal dementia or corticobasal degeneration, and non-specific as dementia with Lewy bodies. The longer-term clinical follow-up was consistent with the post-PET diagnosis in most cases (89%), including in amnestic Aβneg-AD whose post-PET diagnosis remained AD. While the non-amnestic and non-specific Aβneg-AD usually showed patterns of atrophy and hypometabolism suggestive of another
degenerative disorder, the amnestic Aβneg-AD had subtle atrophy and hypometabolism, restricted to the retrosplenium – posterior cingulate – posterior hippocampus junction. Aβneg-AD patients have heterogeneous clinical presentations and likely represent a mixed population of initially misdiagnosed, mostly neurodegenerative, conditions. The clinical, cognitive, MRI and FDG profiles aided to find an alternative post-PET diagnosis in most non-amnestic cases. In the largest and most intriguing subgroup of amnestic Aβneg-AD however, the patients mimic typical AD in their clinical presentation and follow-up, so that an alternative diagnosis was not made in more than half of the cases – highlighting the need for a clinical framework and terminology to define these patients, who may have underlying limbic-predominant, non-Aβ-driven pathologies.
1. Introduction

β-Amyloid (Aβ) deposition is one of the neuropathological hallmarks of Alzheimer’s disease (AD) (Hyman et al., 2012). For more than a decade, it has been possible to visualize these lesions in vivo with positron emission tomography (PET) radiotracers that bind to fibrillar Aβ plaques (Klunk et al., 2004). Most patients with a clinical diagnosis of probable AD have a positive Aβ scan (Aβpos-AD). However, a significant proportion, about 15% (ranging from 2 to 32%) of patients across clinical series, have a negative Aβ scan (Aβneg-AD) (Doraiswamy et al., 2012; Jagust et al., 2010; Ossenkoppele et al., 2013; Ossenkoppele, Jansen, et al., 2015; Rowe et al., 2010; Sperling et al., 2014; Vandenberghe et al., 2010). Very few such cases have come to autopsy so that their etiology remains largely unknown. Some of the Aβneg-AD might correspond to false negatives due to technical issues or scan misinterpretation, or a lack of sensitivity of Aβ ligands in cases with low Aβ burden or atypical Aβ forms (Cairns et al., 2009; Johnson et al., 2013; Rosen et al., 2010; Schöll et al., 2012). The majority of Aβneg-AD cases probably reflect clinical misdiagnosis, as the accuracy of the clinical diagnosis of probable AD at expert centers is approximately 70% when compared to the cause of dementia as determined at autopsy (Beach et al., 2012). Clinical series have shown that clinicians change their diagnosis after disclosure of PET results from AD to a non-Aβ neurodegenerative or non-degenerative condition in a significant portion of Aβneg-AD cases, especially when prior diagnostic certainty was low (Ossenkoppele et al., 2013; Sánchez-Juan et al., 2014). This is particularly the case for patients who present with an atypical (non-amnestic) clinical phenotype (e.g. behavioural-predominant or language deficits). However, clinicians may not revise their diagnosis when faced with a progressive amnestic disorder suggestive of “typical” AD, and identifying the etiologies of these intriguing cases is particularly challenging. In-depth description of the atrophy and hypometabolism pattern and longitudinal clinical trajectories of these patients would further our understanding of their possible underlying pathology, which is crucial to
improve both the clinical diagnosis of AD and AD-like dementia and the understanding of the pathological mechanisms leading to AD symptoms.

In the present study, we gathered detailed clinical and neuroimaging data on Aβneg-AD cases from different samples to further characterize this population compared to Aβpos-AD and Aβ negative healthy controls (Aβneg-HC). Patients were split in subgroups according to their baseline clinical presentation with the two following main objectives: i) to determine the most plausible alternative diagnosis per subgroup based on all available information (clinician judgment based on clinical, neuropsychological, CSF, neuroimaging data and follow-up clinical information); and ii) in the AD-mimic typical amnestic subgroup, especially those without an alternative diagnosis, to provide a comprehensive description of their neuroimaging (atrophy and hypometabolism) profile as a key to the possible etiologies.

2. Methods

2.1. Participants

Aβneg-AD cases were identified by database searches in four Aβ PET research centers. In two centers recruitment for Aβ PET was derived from observational research studies of typical amnestic AD (Caen, France: CAEN and Melbourne, Australia: MEL), whereas in the other two recruitment centered around clinical populations with more diverse clinical profiles (Amsterdam, The Netherlands: AMS and San Francisco, United States: SF). Individuals were eligible for inclusion in this study if they had i) a pre-PET clinical diagnosis of probable AD according to international consensus NINCDS-ADRDA criteria (McKhann et al., 1984) without taking into account imaging data; ii) an Aβ-PET scan that was classified as negative by local readers (VLV, CCR, WJJ, VLS, GDR, BVB); and iii) a structural MRI scan (used for MRI and FDG-PET data processing).
All Aβ-PET scans (PIB or florbetapir standardized uptake value [SUV] images; see Supplementary Table S2) from the four centers were re-reviewed by a single reader blinded to all clinical information (GDR). Ambiguous cases (i.e. high degree of uncertainty or discordance across readers) were excluded, since the goal of this study was to characterize the clearly negative (compared to the clearly positive) AD cases, and not to deal with the issue of intermediate/ambiguous Aβ scans. Out of the 46 Aβneg-AD cases pre-selected by the centers (representing 9%-21% of all AD cases with an Aβ PET scan in those centers), 38 cases were finally included in the present study (Table 1; 3 from CAEN, 6 from MEL, 18 from AMS and 11 from SF). Among the 8 cases that were excluded, 6 had ambiguous or positive Aβ-PET reading on re-review, 1 had an ambiguous pre-PET diagnosis and 1 was too severely impaired. For comparison, Aβ-positive AD cases (Aβpos-AD) and Aβneg-HC from each center were selected. The Aβpos-AD cases were eligible if they had a pre-PET clinical diagnosis of probable AD according to the NINCDS-ADRDA criteria (McKhann et al., 1984) with a typical amnestic clinical presentation, a structural MRI scan and an Aβ-PET scan that was classified as positive by the local reader. The Aβneg-HC were volunteers recruited through newspaper advertisements as described elsewhere (Mevel et al., 2013; Mormino et al., 2009; Ossenkoppele et al., 2012; Villemagne et al., 2011), who performed within normal limits on screening cognitive tests assessing memory, attention, language, visuo-spatial and executive functions. The same reader as for the Aβneg-AD (GDR) performed a blinded review of all Aβpos-AD and Aβneg-HC cases and all cases with an ambiguous Aβ PET scan were excluded. The Aβpos-AD and Aβneg-HC cases were selected so that the groups were matched to the Aβneg-AD group for age and education (and MMSE for AD). In total, 27 Aβpos-AD and 29 Aβneg-HC cases were included in the study. All participants included in this study underwent standard dementia screening that included medical history, informant-based history, physical and neurologic examinations, screening laboratory tests, MRI and neuropsychological testing. Pre-
PET clinical diagnosis was established by consensus in a multidisciplinary team. The demographic characteristics of the groups are indicated in Table 1. All participants or their surrogates provided informed consent to participate in research, and the local ethics committee in each centre approved for all protocols.

2.2. Data collection

To optimize data collection, AP or GC performed site visits at each of the centers following a pre-specified procedure. Before the visit, each centre prepared a list of cases (Aβneg-AD, Aβpos-AD and Aβneg-HC) with their corresponding demographic, ApoE genotype and neuropsychological data, results of CSF analyses when available, and a file summarizing available neuroimaging data (structural MRI, FDG-PET, Aβ-PET).

The procedure for the site visit included:

i) Reviewing the clinical history with one of the local site investigators (who was informed about each case being Aβneg-AD, Aβpos-AD or Aβneg-HC) based on the information available in the clinical report. When information was unclear or missing, the attending clinician of the patient (if different from the site investigator) was further interviewed. The systematically recorded clinical information included: presenting cognitive complaints, date of the first visit, reports from the clinical and neuropsychological assessment, whether the patient had a typical amnestic or non-amnestic presentation, the differential diagnosis (if any), change of diagnosis after disclosing results of the PET scan, clinical follow-up and results of post-mortem analyses if available;

ii) Checking that all neuroimaging data were available and de-identified;

iii) Copying the neuroimaging data and performing a first pass quality control;

iv) Getting information and explanation on the neuropsychological tests and scores.
All Aβneg-AD patients were then classified according to their clinical phenotype in the last assessment prior to the PET scan. The clinical phenotype was determined by the clinician based on clinical and neuropsychological information. They were classified as i) “amnestic” Aβneg-AD if they had predominant episodic memory deficits, with various involvement of other cognitive domains; ii) “non-amnestic” Aβneg-AD if their predominant deficit was in another cognitive domain than memory – i.e. if they had predominant language, visuospatial or frontal symptoms, while memory deficits, if present, were less prominent, or iii) “non-specific” Aβneg-AD if they had a diffuse pattern of cognitive impairment (i.e. they did not present with a predominant deficit in one specific area of cognition).

2.2.1. Neuropsychological scores
To quantify and compare subgroup’s performances, the same or an equivalent test was selected within each center for each of the following cognitive functions: verbal episodic memory (immediate and delayed recall), visual episodic memory, executive functions, visuo-spatial function and semantic memory. The tests and scores selected for each centre are indicated in the Supplementary Material (Table S1). Each score was z-score transformed based on a control database from each corresponding centre.

2.2.2. Neuroimaging data
The scanner types and acquisition protocols for each site are indicated in Supplementary Materials. For voxel-wise analyses, MRI and FDG-PET data were processed and analyzed using SPM5 software (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK). T1-weighted MRI images were segmented, spatially normalized to the MNI space, modulated to correct for nonlinear warping effects using the VBM5.1 toolbox and smoothed using a 12 mm full-width at half-maximum (FWHM) Gaussian kernel. FDG-PET images were co-registered onto corresponding MRI, normalized using the deformation parameters defined from the VBM procedure performed on the corresponding MRI, scaled
using the mean PET value of the cerebellar gray matter and smoothed using a 12 mm FWHM Gaussian kernel.

Each scan was subject to a careful quality check both before and after preprocessing by three neuroimaging experts with more than 10 years of experience in neuroimaging data processing and analyses (FM, BL and GC). MRI data were considered for further analyses if the raw image and the results of the normalization and the segmentation processes were considered to be reliable. For FDG PET data, the selection was based on the quality of the raw PET images and the success of the co-registration (of the PET image onto the corresponding MRI) and the normalization (of the MRI) processes. The selection of images for voxel-wise analyses was based on a consensus agreement from the three experts on the criteria defined above based on qualitative assessment. Note that PET data were not corrected for atrophy as this would rather exacerbate differences due to the different MRI scanners.

79 MRI scans (n= 32 Aβneg-AD, 24 Aβneg-HC and 23 Aβpos-AD) and 72 FDG-PET scans (n= 32 Aβneg-AD, 19 Aβneg-HC and 21 Aβpos-AD) were included in the corresponding voxel-wise analyses. The demographic and clinical characteristics of the respective samples are indicated in Supplementary material; there was no significant difference in the characteristics of the MRI and FDG subsamples compared to those of the main sample.

2.2.3. Cerebrospinal Fluid (CSF)

CSF sampling was obtained in a proportion of the Aβneg-AD (18/38) from AMS and SF. In AMS, CSF was collected in 10 mL polypropylene tubes. Within 2h after collection, the CSF was centrifuged at 1800g for 10 min at 4°C and transferred into a second polypropylene tube, and stored at −20°C. Within 2 months after lumbar puncture, analysis of Aβ-β 1–42 (Aβ1–42), total tau (tau) and tau phosphorylated at threonine-181 (ptau) was performed using sandwich ELISAs (Innotest β-Aβ(1–42), Innotest hTAU-Ag and Innotest Phosphotau(181P); Innogenetics, Gent, Belgium). Aβ 1-42 was considered abnormal <550 pg/ml, total tau > 374
pg/ml and ptau > 52 pg/ml or when the ratio of total tau/Aβ 1-42 was > 0.52 (Duits et al., 2014). Analyses were done by operators who were blinded to all clinical information. CSF collection and processing in SF followed the Alzheimer’s Disease Neuroimaging Initiative (ADNI) protocol (Shaw et al., 2009). Samples were frozen on dry ice and shipped overnight to the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center. Aβ1-42, tau (total), and p-tau181p were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium). Thresholds for CSF biomarkers and biomarker ratios were adopted from the autopsy-proven study by Shaw et al. (Shaw et al., 2009).

### 2.3. Statistical analyses

Demographic, clinical and neuropsychological data were compared between groups using ANOVAs and post-hoc two-by-two group comparisons. Chi-square tests were performed for categorical variables (gender and ApoE4). MRI and FDG-PET images were compared voxel-wise between groups using the full factorial design in SPM5. Results are displayed at a threshold of uncorrected p (p_{unc})<0.001 (cluster extent k>10 voxels) unless specified otherwise. Results described below are presented with all models performed without covariates. This appears as the best option given that the groups were matched to avoid reducing the degrees of freedom and associated statistical power in our analyses. Yet, to ensure that none of our findings were merely reflecting the effects of a covariate, all analyses were repeated including age, gender or centre as a covariate.

### 3. Results

#### 3.1. Demographic and clinical data
The Aβpos-AD, Aβneg-AD, and Aβneg-HC did not differ in age, gender or education (Table 1). The proportion of APOE4 carriers was significantly higher in Aβpos-AD compared to both controls and Aβneg-AD but was not different between the controls and Aβneg-AD.

Table 1: Demographic and clinical characteristics of the samples

<table>
<thead>
<tr>
<th></th>
<th>Aβneg-AD (n=38)</th>
<th>Aβpos-AD (n=27)</th>
<th>Aβneg-HC (n=29)</th>
<th>Group effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>67.4 ± 9.1</td>
<td>70.6 ± 9.8</td>
<td>69.3 ± 11.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>27 : 11</td>
<td>13 : 14</td>
<td>14 : 15</td>
<td>0.09</td>
</tr>
<tr>
<td>Education</td>
<td>12.1 ± 4.1</td>
<td>13.1 ± 3.3</td>
<td>13.5 ± 4.0</td>
<td>0.4</td>
</tr>
<tr>
<td>MMSE</td>
<td>23.0 ± 4.4</td>
<td>23.5 ± 3.3</td>
<td>29.0 ± 1.0*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APOE4 ^1</td>
<td>4/32 (12%)</td>
<td>17/22 (77%)**</td>
<td>3/28 (11%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*: significant difference from both other groups in post-hoc tests (p<0.001); **: significant difference from both other groups in 2x2 Chi-square (p<0.001). ^1indicated is number of APOE4 cases / number of cases with APOE genotyping (proportion).

Among Aβneg-AD patients, there were 19 amnestic, 13 non-amnestic, and 7 non-specific cases. Aβneg-AD subgroups did not differ in age, gender, education or ApoE4 proportion. There was a trend for a group effect on MMSE (p=0.08) and post-hoc analyses showed that non-specific Aβneg-AD had slightly lower MMSE than amnestic Aβneg-AD (Table 2). Comparisons of the neuropsychological scores between these subgroups were consistent with their classification, showing overall more severe episodic memory deficits in Aβpos-AD and amnestic Aβneg-AD, while non-amnestic Aβneg-AD had lower performance on non-memory tasks (see details in Supplemental data text and Figure S1).
Table 2: Demographic and clinical characteristics in Aβneg-AD subgroups

<table>
<thead>
<tr>
<th></th>
<th>Amnestic Aβneg-AD (n=19)</th>
<th>Non-amnestic Aβneg-AD (n=12)</th>
<th>Non-specific Aβneg-AD (n=7)</th>
<th>Group effect (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>68.6 ± 11</td>
<td>64.8 ± 5.7</td>
<td>68.3 ± 8.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>12 : 7</td>
<td>9 : 3</td>
<td>6 : 1</td>
<td>0.5</td>
</tr>
<tr>
<td>Education</td>
<td>11.9 ± 4.5</td>
<td>12.6 ± 3.6</td>
<td>10.5 ± 2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>MMSE</td>
<td>24.5 ± 2.6</td>
<td>21.0 ± 5.9*</td>
<td>22.6 ± 4.8</td>
<td>0.08</td>
</tr>
<tr>
<td>APOE4 1</td>
<td>4/17 (24%)</td>
<td>0/11 (0%)</td>
<td>0/6 (0%)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*: post-hoc Fisher LSD difference from amnestic Aβneg-AD p=0.04. 1indicated is number of APOE4 cases / number of cases with APOE genotyping (proportion).

After Aβ PET, the clinicians altered the diagnosis in 23 of 34 (68%; missing information in 4 cases) Aβneg-AD cases (Figure 1). Post-Aβ PET diagnoses included behavioral and/or language variants of frontotemporal dementia (FTD, n=11), corticobasal degeneration (CBD, n=5), dementia with Lewy bodies (DLB, n=3), epilepsy/depression (n=1), hippocampal sclerosis (n=1) and unknown (n=2, see Figure 1). The FTD diagnoses included non-fluent variant of primary progressive aphasia (n=3), behavioural variant of FTD (n=2), semantic dementia (n=1), mixed language and behavioral FTD (n=2), unspecified variant (n=2) and atypical FTD (n=1).

When considering Aβneg-AD subgroups (Figure 1), clinicians did not change their clinical diagnosis in 56% of amnestic Aβneg-AD patients, but nearly always changed the diagnosis in non-amnestic and non-specific cases (94%; significantly different from the percentage in amnestic Aβneg-AD; chi-square p=0.002). Amnestic Aβneg-AD cases were most often reclassified as FTD, non-amnestic as FTD or CBD, and non-specific as DLB.
Among the patients who had longer-term (>2 years) follow-up (n=27, 80%), the post-PET diagnosis was supported and remained unchanged in most cases (n=24; 89%). Three non-amnestic Aβneg-AD patients were followed to death and underwent brain autopsy at the UCSF Neurodegenerative Disease Brain Bank following previously published protocols (Ossenkoppele, Pijnenburg, et al., 2015). The post-mortem diagnoses were corticobasal degeneration (2) and Pick’s disease, pathological variants of frontotemporal lobar degeneration (Mackenzie et al., 2010). The two former cases had no amyloid at all (Thal stage 0, CERAD absent) and the later case showed sparse diffuse plaques without neuritic plaque (Thal stage 1, CERAD absent).

**Figure 1:** Clinical diagnosis at baseline (in the last assessment prior to the Aβ-PET scan; 1st line), once the clinicians knew the results of the PET scan (2nd line), and after a >2 years clinical follow-up (3rd line). The neuropathological diagnosis is also indicated in three patients who died and underwent autopsy (4th line). AD: Alzheimer’s disease; CBD: corticobasal degeneration; CBD: corticobasal degeneration; DLB: dementia with Lewy Body; Ep: Epilepsy-depression; FTD: frontotemporal dementia; HS: hippocampal sclerosis; MCI unkn: MCI with unknown etiology; Prb: probable; unkn.: unknown disease.
3.2. CSF biomarkers

The results of CSF AD biomarkers in Aβneg-AD are indicated in Table 3. CSF Aβ42 results in Aβneg-AD patients were usually in the normal range, concordant with the negative Aβ PET. However, CSF total Tau or p-Tau levels were abnormal in more than half the cases. Only one patient had a CSF profile strongly suggestive of underlying AD, with low Aβ42 and high Tau/p-Tau. The results of Aβneg-AD subgroups should be considered with caution because of the small sample sizes. They indicate that amnestic Aβneg-AD had either i) normal CSF results (45%) or ii) normal CSF Aβ42 with increased CSF total Tau or p-Tau levels (55%). Non-amnestic Aβneg-AD presented with any combination of normal or abnormal Aβ42 or Tau/p-Tau levels; they notably included all Aβneg-AD cases with decreased Aβ42. CSF results were obtained in only 3 non-specific Aβneg-AD and 2 had increased Tau or p-Tau levels with normal Aβ42.

Table 3: CSF profile per Aβneg-AD subgroup: number of cases (percentage)

<table>
<thead>
<tr>
<th></th>
<th>N total</th>
<th>Aβ42 normal, Tau/p-Tau normal</th>
<th>Aβ42 low, Tau/p-Tau normal</th>
<th>Aβ42 normal, Tau/p-Tau high</th>
<th>Aβ42 low, Tau/p-Tau high</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>18</td>
<td>7 (39%)</td>
<td>1 (5.5%)</td>
<td>9 (50%)</td>
<td>1 (5.5%)</td>
</tr>
<tr>
<td>Amnestic Aβneg-AD</td>
<td>9</td>
<td>4</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Non-amnestic Aβneg-AD</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Non-specific Aβneg-AD</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>
3.3. Neuroimaging

The neuroimaging findings in the total Aβneg-AD group, compared to Aβpos-AD and Aβneg-HC, are described in the Supplemental data (text and Figure S2). The neuroimaging findings for the different Aβneg-AD subgroups are shown in Figure 2.

Compared to Aβneg-HC, significant atrophy in amnestic Aβneg-AD was restricted to small clusters in the right and left retrosplenium (not surviving family-wise correction for multiple comparisons at pFWE<0.05). With a more permissive threshold (p_{unc}<0.005), atrophy was also found in the hippocampus (anterior and posterior portions), ventral posterior cingulate cortex and orbito-frontal and dorsomedial prefrontal cortex. There was no area of significant hypometabolism in amnestic Aβneg-AD compared to Aβneg-HC; even at a more permissive threshold (p_{unc}<0.005), only very small clusters in the medial prefrontal, right middle temporal and posterior cingulate cortex were observed. As expected, both atrophy and hypometabolism were significantly less pronounced in amnestic Aβneg-AD compared to Aβpos-AD, in the temporo-parietal cortex (surviving at pFWE<0.05 in the left side) and precuneus.

In non-amnestic Aβneg-AD, asymmetric atrophy was found in left greater than right prefrontal, temporal, temporoparietal and temporo-occipital cortex, temporal pole, insula, posterior cingulate and precuneus, amygdala and parahippocampal gyrus. The hippocampus was mostly preserved (except a small portion in the posterior end of the right hippocampus). Large portions of the prefrontal cortex and small clusters in the left temporal lobe survived multiple comparisons correction (pFWE<0.05). Significant hypometabolism was found in left greater than right dorsal (mainly lateral) prefrontal cortex and left angular gyrus (both surviving at pFWE<0.05). Compared to Aβpos-AD, non-amnestic Aβneg-AD showed greater atrophy especially in frontal and insular regions and caudate nucleus, but they also had small clusters of less significant atrophy in posterior temporal cortex. No significant difference was found in hypometabolism between non-amnestic Aβneg-AD and Aβpos-AD.
Compared to Aβneg-HC, the non-specific Aβneg-AD showed restricted areas of atrophy in the orbital and dorsomedial frontal cortex (not surviving at pFWE<0.05), and significant hypometabolism predominantly in the temporal neocortex (surviving at pFWE<0.05) extending to the temporoparietal junction, and the prefrontal cortex (mainly bilateral). Compared to Aβpos-AD, atrophy was slightly less pronounced in non-specific Aβneg-AD while hypometabolism was more pronounced in the left insula and bilateral lingual cortex.
Figure 2: Profiles of atrophy (A) and hypometabolism (B) in the three Aβneg-AD subgroups and in the Aβpos-AD compared to the Aβneg-HC. The threshold was set at $p_{unc}<0.001$, $k>10$. 
3.4. Additional analyses in the amnestic Aβneg-AD subgroup

To further understand what distinguished amnestic Aβneg-AD from Aβpos-AD cases, we split Aβneg-AD according to their post-PET diagnosis, i.e. whether or not the diagnosis changed after the clinician knew the results of the Aβ-PET scan. The diagnosis did not change in 10 amnestic Aβneg-AD (i.e. Aβneg-AD-unchanged) (Figure 1). Within this subgroup, longer term (> 2 years) follow-up was available in all but one patient, and the diagnosis remained probable AD in 7/9 cases while diagnosis changed to unspecified MCI in the two remaining cases because their functional impairment was in the gray zone between MCI and dementia and they did not deteriorate during the follow-up. The 8 amnestic Aβneg-AD cases with a post-PET change in diagnosis were called amnestic Aβneg-AD-changed. Longer term (> 2 years) follow-up was available in 6 out of these 8 patients and the clinical diagnosis remained the same as the post-PET diagnosis in all cases.

The neuroimaging findings of the amnestic Aβneg-AD subgroups are shown in Figure 3. Because of the limited size of the subsamples, all results are shown and described at \( p_{unc}<0.005 \) (and \( p_{unc}<0.05 \) when specified) with cluster extend \( k>50 \) voxels. The amnestic Aβneg-AD-unchanged showed significant atrophy and hypometabolism compared to Aβneg-HC in the same region of the retrosplenial cortex encroaching the posterior hippocampus as amnestic Aβneg-AD. Interestingly, even at an exploratory threshold of \( p_{unc}<0.05 \), atrophy and hypometabolism remained mainly constrained to the posterior hippocampus, posterior cingulate and precuneus.

The amnestic Aβneg-AD-changed group had significantly more atrophy compared to Aβneg-HC in the medial orbitofrontal cortex, dorso-medial frontal cortex (superior frontal gyrus), thalamus, amygdala, and parahippocampal gyrus. Significant hypometabolism was observed in the middle and superior temporal gyri.
Individual profiles of atrophy and hypometabolism were also assessed as illustrated in Supplemental material (Figure S3). They showed that three different scenarios could be found amongst the amnestic Aβneg-AD. About half of the cases presented with very slight and similar profiles of atrophy and hypometabolism restricted to the posterior hippocampus, retrosplenium and/or posterior cingulate cortex (representative examples in Figure S3B and S3C). In these cases, the clinical follow-up did not allow to identify an alternative diagnosis to probable AD.

About one third of the cases had a profile of atrophy and hypometabolism consistent with another degenerative disease (representative example in Figure S3F); the clinicians changed the diagnosis based on this information and in all cases the longer-term clinical evolution was consistent with the new diagnosis. Finally, a small proportion of the amnestic Aβneg-AD cases (n=4, 22%) had a clinical progression that was not consistent with a neurodegenerative disease in that they were relatively stable or declined very slowly. Interestingly, the profiles of atrophy and hypometabolism were different in these later cases compared to both previous scenarios, in that they had either no atrophy and no hypometabolism, or very discrepant profiles with pronounced and extended atrophy and almost no hypometabolism (Figure S3D and S3E). It seems relevant to identify these cases as their clinical outcome is different and they likely do not have a neurodegenerative disease.
Figure 3: Profiles of atrophy (upper row) and hypometabolism (lower row) in Aβpos-AD (left), amnestic Aβneg-AD-unchanged (middle) and amnestic Aβneg-AD changed (right), compared to Aβneg-HC. The results are displayed at $p_{unc}<0.005$ and $p_{unc}<0.05$, $k>50$; scale is in T-values.

4. Discussion

In this multicenter study we assessed the clinical, neuropsychological and neuroimaging features of patients clinically diagnosed with Alzheimer’s disease who had an amyloid-negative PET scan. We found Aβneg-AD patients to have heterogeneous clinical presentations and outcomes. 50% had a clinical phenotype typical of AD with memory predominant deficits (amnestic Aβneg-AD), 32% showed an atypical presentation with predominant deficits in a non-memory domain (non-amnestic Aβneg-AD), while the remaining 18% had a non-specific neurobehavioral phenotype (non-specific Aβneg-AD). After disclosure of PET scan results, the diagnosis was changed in two-thirds of all cases, including 44% of amnestic-Aβneg-AD cases versus all but one (94%) of non-amnestic and non-specific cases. The alternative diagnosis was
another degenerative condition in a majority of cases (56% of all Aβneg-AD cases, 87% of the non-amnestic and non-specific Aβneg-AD cases), which reflects the overlap in clinical expression between the different degenerative diseases. The most frequent alternative diagnoses were FTD (48% of the cases for which the diagnosis was changed), CBD (22%) and DLB (13%).

In the national Alzheimer’s coordinating center (NACC) autopsy database, a mismatch between the clinical and neuropathological diagnoses of AD was found in 17% of the 526 subjects diagnosed as clinically probable AD (Beach et al., 2012), and in 25% of patients diagnosed with possible or probable AD in a follow-up study (Monsell et al., 2015). The proportion of Aβneg-AD cases in the four centers in the present study (9 to 21%) is comparable to these postmortem studies, and to the rate of clinically diagnosed AD patients with negative Aβ PET reported in the literature (Doraiswamy et al., 2012; Jagust et al., 2010; Ossenkoppele, Jansen, et al., 2015; Salloway et al., 2014; Vandenberghe et al., 2010).

The most frequent primary neuropathological diagnoses for the cases not meeting the neuropathological threshold for AD in Beach et al. (2012) were AD nevertheless (19%), FTD (17%; amongst which 7/15 had ubiquitin or TDP-43 positive inclusions and 3/15 had tauopathies), tangle-only dementia or argyrophilic grain disease (17%), cerebrovascular disease (11%), DLB (10%), hippocampal sclerosis (9%) and CBD (2%). The alternative clinical diagnoses in the present study were mostly similar, with differences likely reflecting the differences in the study design (e.g. postmortem versus clinical diagnoses, availability of both plaque and tangle data at autopsy versus Aβ biomarker only in the present study).

A proportion of Aβneg-AD might reflect false negative Aβ scans. However, the fact that Aβneg-AD showed different profiles of hypometabolism and atrophy as compared to Aβpos-AD makes this an unlikely explanation in the majority of cases in this study. Moreover, most Aβneg-AD had a normal CSF level of Aβ42, consistent with previous reports (Shimada et al.,
2011; Takeuchi et al., 2012), and studies showing high agreement between amyloid PET and CSF Aβ results. (e.g. (Palmqvist et al., 2014; Zwan et al., 2014)). Only two patients had low CSF Aβ42, suggesting that false negative Aβ PET may occur infrequently at least in our cohort, although postmortem confirmation would be needed. A few cases (especially those with an AD-typical phenotype and clinical evolution, or low CSF Aβ) might yet have low levels (Cairns et al., 2009; Leinonen et al., 2008) or an atypical form (Schöll et al., 2009) of Aβ, that would not be detected with Aβ PET. As regard to CSF Tau and p-Tau, the high levels found in about half of the cases indicates that neurodegeneration and/or neurofibrillary tangles are likely present in at least 50% of Aβneg-AD in our study (Blennow et al., 2010).

Aβneg-AD patients were characterized by a low prevalence of APOE4 (12% against 77% in the Aβpos-AD), consistent with previous reports (Serrano-Pozo et al., 2014; Shimada et al., 2011; Takeuchi et al., 2012) and with the fact that APOE4 is strongly associated with Aβ deposition (Fouquet et al., 2014). In Monsell et al. (2015), minimal plaques were found postmortem in 13% of APOE4 carriers versus 37% of non-carriers in patients with a clinical diagnosis of possible or probable AD. In a recent clinical trial of anti-Aβ immunotherapy, the prevalence of Aβ PET-negativity in patients clinically diagnosed with mild-moderate AD dementia was 6.5% in ApoE4 carriers versus 36% in non-carriers (Liu et al., 2015; Salloway et al., 2014).

4.1. Amnestic Aβneg-AD

The largest subgroup of Aβneg-AD patients presented with a progressive amnestic disorder consistent with typical AD, and performed most similarly to Aβpos-AD on cognitive tests. The clinical follow up suggests that in most cases this condition is not benign: only 3/15 patients with longer-term clinical follow-up were reclassified as MCI as their cognition remained stable, while the others showed clinical progression consistent with ongoing neurodegeneration and dementia (i.e. probable AD in 7, FTD in 3, hippocampal sclerosis and DLB in 1 each). Within
this group, patients whose diagnosis changed after the Aβ PET scan (44%) were most often reclassified as FTD (in 50% of the cases), and their neuroimaging profiles consistently showed predominant fronto-temporal alterations. However the diagnosis remained unchanged in 56% of cases. In the Aβneg-AD-unchanged group, atrophy and hypometabolism were restricted to the hippocampus, retrosplenial and PCC areas. These regions are known to be highly connected and involved in episodic memory (see e.g. (Ranganath and Ritchey, 2012)), which is consistent with the predominant episodic memory deficits of these patients. These patients seem likely to harbour a variety of limbic-predominant pathologies affecting the medial temporal lobe. One likely cause may be tangle-predominant dementia. Along the line of the recently termed primary age-related tauopathy (PART), patients with a clinical diagnosis of AD and neurofibrillary tangles but lacking Aβ plaques have been described in many cohorts (Crary et al., 2014). Amongst clinically diagnosed AD cases with no or sparse neuritic plaques from autopsy (excluding the cases with a non-AD pathological diagnosis in Serrano-Pozo et al., 2014), 40 to 45% had substantial neurofibrillary degeneration (Braak stages ≥ III) (Monsell et al., 2015; Serrano-Pozo et al., 2014). On the other hand, more than half of Aβ-negative patients thus had Braak stages 0/I/II of neurofibrillary tangles, which is insufficient to account for their mild-to-moderate dementia. Additional neuropathologies that specifically target the medial temporal lobe and hippocampal circuit include hippocampal sclerosis (with or without TDP-43 positive inclusions (Nag et al., 2015)) and argyrophillic grain disease, a primary tauopathy with inclusions that are morphologically and biochemically distinct from neurofibrillary tangles (Grinberg et al., 2013). Cerebrovascular disease and dementia with Lewy bodies can also mimic typical AD clinically, though are more often associated with a non-amnestic predominant clinical phenotype. Notably, Serrano-Pozo and colleagues found essentially no difference in the frequency and severity of concurrent vascular and Lewy body pathologies at autopsy in low versus high amyloid brains of patients diagnosed clinically with AD. White matter lesions were
not assessed in the present study because of the lack of homogeneous data/information across centers. However, clinicians had access to the clinical MRIs and did not make the diagnosis of vascular dementia or vascular MCI despite the negative amyloid scans. Emerging tau-specific PET ligands may shed further light on the underlying pathology in these patients (Villemagne et al., 2015).

It is particularly striking that amnestic Aβneg-AD-unchanged were comparable to Aβpos-AD in their clinical presentation and trajectories, while they had significantly less atrophy and hypometabolism in extended neocortical brain areas. It is possible that these patients have another pathological process, which is not measured here but contributes to their clinical profile and evolution. One can speculate that there is a causal relationship between the lack of amyloid deposition and the lack of atrophy/hypometabolism beyond the hippocampo-posterior cingulate cortex area in Aβneg-AD patients. Thus, Aβ may facilitate the spread of pathologies (e.g. tau in AD) and related neurodegeneration from the initial site of infection to distant connected brain regions (i.e. temporo-parietal, precuneus and frontal areas in AD). On the same line, as Aβneg-AD tend to show very similar patterns of atrophy and hypometabolism, our results raise the question of the role of Aβ in the mismatch between atrophy and hypometabolism patterns typically found in AD (Chételat et al., 2008; La Joie et al., 2012).

4.2. Non-amnestic and non-specific Aβneg-AD

A second group of Aβneg-AD patients was characterized by non-amnestic predominant clinical presentations. These patients showed relatively greater impairment in non-memory domains compared to Aβpos-AD and amnestic Aβneg-AD. Predominant deficits in language, executive functions/behavior and visuospatial function characterize ~15% of AD patients presenting to academic dementia centers (Snowden et al., 2007) and even more in early-onset AD (Mendez
et al., 2012). While these presentations are now recognized as AD phenotypes and are included in newly proposed AD diagnostic criteria (Dubois et al., 2014; McKhann et al., 2011), these patients also show significant clinical overlap with FTD-spectrum disorders (Alladi et al., 2007; Ossenkoppele, Pijnenburg, et al., 2015). In these cases clinicians changed their clinical diagnosis to FTD-spectrum syndromes (such as behavioral variant FTD, non-fluent variant primary progressive aphasia or CBD), and the topography of atrophy and hypometabolism was consistent with the alterations typically found in FTD (Diehl et al., 2004; Rabinovici et al., 2007), CBD (Lee et al., 2011), and primary progressive aphasia (Gorno-Tempini et al., 2011; Nestor et al., 2003; Rabinovici et al., 2008). These diagnoses remained stable over time.

The third (and smallest) subtype of Aβneg-AD presented with non-specific clinical symptoms and cognitive deficits. In these patients, AD may have represented a “default” diagnosis for a condition felt to be neurodegenerative in origin, but failing to conform a clearly described cognitive-behavioral syndrome. This group did not show a clear “signature” in the post-PET diagnoses (including DLB, CBD, and unknown dementia), clinical evolution, cognitive testing or MRI/FDG patterns, reflecting its heterogeneity as well as small numbers. In addition to cognitive deficits, these patients often have one or more of the following: global slowing (2 cases), parkinsonism (1 case), depression (1 case), vascular lesions (2 cases), hallucinations (2 cases) and cognitive fluctuations (2 cases), representing a mix of core DLB features, as well as potential non-degenerative comorbidities that might impact cognition. Indeed, in two cases cognition was stable or even improved at follow-up, suggesting that some non-specific patients, despite meeting criteria for dementia at one point, may not have an underlying neurodegenerative disease. This subtype illustrates the utility of Aβ PET for “ruling-out” AD in patients with non-specific presentations, and potentially identifying treatable non-degenerative etiologies in a subset.

4.3. Limitations
The lack of autopsy data (except in three cases) is a limitation of the present study as postmortem analysis would be the gold standard for identifying the etiology of Aβneg-AD cases. Note that 19% of the cases not meeting full neuropathological criteria for AD in Beach et al. (2012) were nevertheless diagnosed with AD as the primary cause of dementia, illustrating that histopathological analyses does not always provide a clear answer; in some cases, the pathological processes underlying their dementia might not be identified using current techniques.

Another limitation is the lack of standard cognitive tests and the fact that we compared retrospectively data from different centers that sometimes used different cognitive tests. Similarly, only clinical follow-up was available in the present study. Future prospective, longitudinal studies including an Aβneg-AD sample tested using a standardized neuropsychological battery will be needed to further assess whether subtle difference in the nature, degree or evolution of cognitive (including episodic memory) deficits are present.

4.4. Conclusion

This study shows that Aβneg-AD is neither a rare nor a benign condition. The clinical evolution suggests an underlying neurodegenerative disease in most patients, including those with a typical amnestic presentation or the less typical non-amnestic cases. In the latter, who likely reflect misdiagnosis, Aβ PET imaging proved to be useful to rule-out AD, as shown in previous studies on the clinical impact of amyloid PET imaging. The individual profiles of atrophy and hypometabolism help, not only to find an alternative diagnosis in those cases, but also to detect the cases that might not have a neurodegenerative disease and remain relatively stable clinically. In the amnestic Aβneg-AD cases however, an alternative diagnosis could not be found in slightly more than half of the cases: they have no amyloid and showed atrophy and hypometabolism restricted to the restrosplenial cortex, but they mimic AD in their clinical presentation as well as in their clinical trajectory. Based on the current neuropathological
definition of AD they should not be called AD, but there is a need for a clinical framework and
terminology for the classification of these patients. They likely represent a mixed population of
limbic-predominant AD-mimics (e.g. tangle-only dementia but also hippocampal sclerosis with
TDP-43 inclusions, or argyrophilic grain disease) or other non-Aβ-driven pathologies. Further
in-vivo exploration (including Tau-PET imaging) and extensive longitudinal assessment with
autopsy data are needed to expand on our understanding of these intriguing clinical cases.

Funding:

This study was supported by the Fondation Plan Alzheimer, Programme Hospitalier de
Recherche Clinique, Agence Nationale de la Recherche, Région Basse Normandie, Institut
National de la Santé et de la Recherche Médicale, NIA P01-AG1972403 (BLM), and ADRC
P50-AG023501 (BLM and GDR), Alzheimer’s Association (GDR), State of California DHS
04-33516 (BLM), John Douglas French Alzheimer’s Foundation (BLM and GDR), Avid
Radiopharmaceuticals (GDR), NHMRC project grant 1071430 (VLV and CCR) and Senior
Research Fellowship (VLV).

Acknowledgements

Authors are grateful to J. Mutlu, C. Tomadesso, R. de Florès, J. Gonneaud, G. Poisnel, S. Egret,
C. Lebouleux, A. Manrique, M-C. Onfroy, L. Barré, A. Abbas, A. Quillard, for their help with
recruitment, cognitive testing and imaging examinations. We thank J. Vogel, P. Ghosh, B.
Cohn-Sheehy, for their help with data collection.

Tables and Figures

Included in the text for the sake of proof reading
References


