The effect of Lewy body (co-)pathology on the clinical and imaging phenotype of amnestic patients

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Abstract

Lewy body (LB) pathology is present as a co-pathology in approximately 50% of Alzheimer's disease (AD) dementia patients and may even represent the main neuropathologic substrate in a subset of patients with amnestic impairments. However, the degree to which LB pathology affects the neurodegenerative course and clinical phenotype in amnestic patients is not well understood. Recently developed α-synuclein seed amplification assays (αSyn-SAAs) provide a unique opportunity for further investigating the complex interplay between AD and LB pathology in shaping heterogeneous regional neurodegeneration patterns and clinical trajectories among amnestic patients.

We studied 865 patients from the ADNI cohort with clinical diagnoses of aMCI (N=661) or AD dementia (N=211), who had CSF and FDG-PET data available. CSF samples were analyzed for peptide levels of Aβ1-42 and p-tau181, and αSyn positivity was evaluated using a novel αSyn-SAA. Based on positive/negative results on the different biomarkers, subjects were classified as "AD-LB-" (N=304), "AD+LB-" (N=335), "AD+LB+" (N=158) and "AD-LB+" (N=68). We analyzed group differences in regional FDG-PET patterns, demographics, APOE4 genotype, baseline and longitudinal domain-specific cognitive profiles (memory vs executive function/visuospatial performance), as well as risk for developing hallucinations.

AD+LB+ showed worse global cognition (MMSE: $p=0.005$) and declined faster ($p<0.001$) than AD+LB-, but both groups exhibited similar memory-predominant cognitive profiles. In FDG-PET,

AD+LB+ showed more severe hypometabolism compared to AD+LB-, but both groups were characterized by largely identical patterns of temporo-parietal hypometabolism. By contrast, AD-LB+ were less globally impaired ($p<0.001$) but characterized by a markedly more dysexecutive and visuospatial profile ($p<0.003$) and a strikingly different posterior-occipital pattern of hypometabolism. APOE4 positivity was similar between AD+LB+ and AD+LB- (72% vs. 75%, $p=0.28$) but lower in AD-LB+ (28%, $p<0.001$). On a group level, AD+LB+, AD+LB-, and AD-LB+ showed similar risks of developing hallucinations, but patients with a LB-like posterior-occipital hypometabolism pattern had a significantly higher risk compared to those showing an AD-typical temporo-parietal pattern (HR=2.58, $p=0.004$).

In conclusion, LB co-pathology in AD was associated with more severe hypometabolism and faster cognitive decline, but did not affect the regional hypometabolic pattern or cognitive profile. By contrast, patients with relatively pure LB pathology showed a more executive/visuospatial-predominant cognitive profile and a distinct posterior-occipital hypometabolism pattern characteristic for LB disease. These findings indicate that the presence of LB pathology may have different consequences for the clinical phenotype depending on AD co-morbidity, which may have critical implications for accurate diagnosis and prognosis of patients presenting with amnestic syndromes.

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Introduction

In Alzheimer's disease (AD), the accumulation of amyloid-β plaques and tau neurofibrillary tangles in the brain typically leads to a characteristic pattern of progressive temporo-parietal neurodegeneration,[1] which is linked to initial memory deficits that progress into a characteristic amnestic dementia syndrome.[2,3] By contrast, Lewy body (LB) pathology, composed of misfolded alpha-synuclein, is typically associated to a distinct dementia syndrome characterized by a relatively more dysexecutive cognitive profile and a higher prevalence of neuropsychiatric abnormalities such as hallucinations.[4] In neuroimaging assessments, dementia with Lewy bodies (DLB) typically shows a distinct pattern of posterior-occipital hypometabolism with a relative sparing of the medial temporal lobe (MTL),[5] which is used in the clinic as a supportive imaging biomarker for the differential diagnosis between AD and DLB.[6,7] However, LB pathology is also present as a co-pathology in ~50% of AD dementia patients,[8–10] and it may even represent the main neuropathologic substrate in a subset of patients with amnestic impairments that are clinically diagnosed as amnestic mild cognitive impairment (aMCI) or AD dementia (ADD).[11–15]

The degree to which LB pathology affects the neurodegenerative course and clinical phenotype in amnestic patients is not well understood. While several clinico-pathologic studies have indicated

that co-morbid LB pathology in AD is associated with faster clinical decline and a younger age of death,[16–18] some have also suggested that it might lead to the development of a mixed dementia phenotype with a more prominent expression of clinical features characteristic for DLB.[9,10,19–22] However, these findings could not be confirmed by others.[17,23,24] In this regard, in a recent imaging-neuropathological study we found that neuropathologically-confirmed AD patients with LB co-pathology (AD-LB) displayed a similar memory-predominant cognitive profile as AD patients without LB co-pathology.[14] Moreover, on ante-mortem FDG-PET imaging, these patients showed the same AD-typical temporo-parietal pattern of hypometabolism as the AD patients without LB co-pathology. By contrast, a small subset of the examined cohort of amnestic patients showed relatively pure LB pathology without meeting pathologic criteria for AD, and these patients did exhibit the DLB-typical posterior-occipital FDG-PET pattern. Interestingly, in individual-level analyses this DLB-typical posterior-occipital hypometabolic pattern was also observed in a small subset of AD-LB patients who showed additional pathological signs of LB-related neurodegeneration, such as substantia nigra neuronal loss at autopsy.[14] To better understand the clinical relevance of this DLB-typical FDG-PET pattern in patients with amnestic presentations typical for AD, in a subsequent study we examined individual FDG-PET patterns in a large in-vivo cohort of >1000 patients with clinical diagnoses of ADD or aMCI. Here we found that approximately 13% of this population displayed a DLB-typical posterior-occipital FDG-PET pattern, and these patients also showed specific clinical features typically associated with LB pathology, such as a more dysexecutive cognitive profile relative to the memory deficit and a significantly higher risk of developing hallucinations over follow-up.[25] Interestingly, these patients also showed significantly less abnormal biomarkers of AD pathology, and particularly tau pathology, indicating that other pathologic factors, presumably LB pathology, may have a stronger contribution to the observed phenotype in these patients. However, validated biomarkers for LB pathology were not yet available at that time and thus the underlying pathologic features of these patients remained unknown in that in-vivo study.

Recently developed alpha-synuclein (αSyn) seed amplification assays (αSyn-SAAs) that detect aggregates of misfolded αSyn in cerebrospinal fluid (CSF) have shown great promise as diagnostic tests for the biochemical diagnosis of LB diseases such as Parkinson's Disease (PD) and DLB,[26] and most recently also for the detection of LB (co-)pathology in the context of clinical AD.[27] This provides a unique opportunity for further investigating the complex interplay between underlying

neuropathology, regional patterns of neurodegeneration, and heterogenous clinical presentations and progression trajectories in amnestic patients. In this study, we used available αSyn-SAA data of a large sample of patients clinically diagnosed with aMCI or ADD to evaluate the effect of LB pathology and its interaction with concomitant AD pathology on the expression of differentiated hypometabolism patterns and clinical trajectories in the context of these amnestic syndromes.

Materials and methods

Study participants

Data used in the preparation for this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI).[28] ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to evaluate whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. All ADNI studies are conducted according to the Good Clinical Practice guidelines, the Declaration of Helsinki, and U.S. 21 CFR Part 50 (Protection of Human Subjects), and Part 56 (Institutional Review Boards). Written informed consent was obtained from all participants before protocol-specific procedures were performed. The ADNI protocol was approved by the Institutional Review Boards of all the participating institutions.

We studied 868 ADNI participants with a baseline diagnosis of aMCI (N=662) or ADD (N=206) who had undergone lumbar puncture and FDG-PET imaging at least once over the course of the ADNI study (query date: January 2024). aMCI was diagnosed according to Petersen criteria, including impaired logical memory according to the Wechsler Memory Scale Logical Memory II, Mini-Mental State Examination (MMSE) between 24 and 30, and a clinical dementia rating (CDR) of 0.5.[29] ADD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for "probable AD",[30] and at study entry patients had to have an MMSE score between 20 and 26, a CDR of 0.5 or 1, and objective evidence of impaired logical memory

impairment. For comparison, we also made use of normative FDG-PET data from 179 cognitively normal elderly ADNI participants (the "healthy *control group*").

Molecular biomarkers and subject stratification

Available antemortem CSF samples from the in-vivo cohort were analysed for peptide levels of $A\beta_{1-42}$ and p-tau181 with the fully automated Roche Elecsys electrochemiluminescence immunoassays on a cobas e601 instrument (Roche Diagnostics, Indianapolis, IN) according to the kit manufacturer's instructions. αSyn-SAAs were performed in the Amprion Clinical Laboratory (CLIA ID No. 05D2209417; CAP No. 8168002) using a method validated for clinical use in accordance with Clinical Laboratory Improvement Amendment (CLIA) requirements.[26] Previously established cut-offs for $A\beta_{1-42}$ (<1097 pg/mL) and p-tau181 (>19 pg/mL) were used to classify the subjects as amyloid positive/negative (A+/-) and tau positive/negative (T+/-).[31] Patients under the lower technical limit of 200 pg/mL for $A\beta_{1-42}$ were classified as A+. Regarding αSyn, the employed SAA provides the results already binarized as "Detected-1" (αSyn aggregates detected, aggregation profile consistent with Type 1 seeds compatible with Parkinson's disease and DLB); "Detected-2" (αSyn aggregates detected, aggregation profile consistent with Type 2 seeds as seen in Multiple System Atrophy); or "Not Detected" (αSyn aggregates not detected). For the purposes of this study, patients presenting "Detected-0" were considered as αSyn-, patients presenting "Detected-1" were considered as αSyn+ and patients presenting "Detected-2" were excluded. Based on positive/negative results on the different biomarkers, subjects were grouped into "AD-LB-", "AD+LB-", "AD+LB+", and "AD-LB+", where a positive AD status required positivity in both for $A\beta_{1-42}$ and p-tau181 biomarkers.[32]

Neuropsychological, clinical, and neuropsychiatric data

All patients included in the final cohort had baseline and longitudinal cognitive assessment data available (average follow-up: 1.7 ± 1.1 years for ADD, 4.7 ± 3.2 years for aMCI). The Mini-Mental State Examination (MMSE) scores were used for characterizing global cognitive performance,[33] and previously established domain-specific composite scores were used for assessing memory (ADNI-MEM)[34], executive function (ADNI-EF)[35], and visuospatial deficits (ADNI-VS).[36] Additionally, two "cognitive profile" variables, Δ(MEM-EF) and Δ(MEM-VS),

were calculated to characterize disproportionate impairments between memory function and the other cognitive domains. These variables were calculated as the difference between ADNI-EF or ADNI-VS and the ADNI-MEM composite scores. Given that the different composite scores are not measured on the same scale, z-scoring (based on the whole sample) was performed before calculation, such that all variables had an equal mean of zero and a standard deviation of one within this sample.[37]

Although core DLB features are not specifically assessed in ADNI, experienced neurologists at the participating memory centers review and update the clinical diagnoses at annual follow-up visits. The available diagnostic information recollects whether the "suspected cause of dementia" is "AD" or "other etiology". Cases with "dementia due to AD" are further classified into "possible AD" or "probable AD" according to clinical likelihood (note that due to inclusion criteria, all ADD subjects have "probable AD" at baseline). We used this diagnostic information to study how the clinical diagnoses of ADD patients and aMCI to dementia converters in the different biomarker-defined groups evolved over the clinical follow-up period. Additionally, participants are longitudinally screened using the Neuropsychiatric Inventory–Questionnaire (NPI-Q),[38,39] in which the participant's caregivers are asked to evaluate the presence and severity of commonly encountered neuropsychiatric symptoms, including hallucinations. NPI-Q was available for all the patients included in our study group. Patients were considered to have developed hallucinations when the caregivers reported their presence according to the NPI-Q criteria, irrespectively of the reported severity. This data was used to assess the emergence of hallucinations over follow-up.

Genetics

APOE genotype was determined by Cogenics using standard methods to genotype the two APOE-ε4-defining SNPs (rs429358, rs7412). Patients were labelled as having zero, one, or two ε4 copies.

FDG-PET imaging

FDG-PET images were acquired by using dynamic 3D acquisitions of six 5-min frames starting 30 minutes after the injection of 185 MBq of FDG. For this work, we used images in pre-processing level four as described by ADNI (adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/), corresponding to co-registered and averaged images of the six frames standardized and smoothed

to a uniform 8 mm isotropic resolution. FDG-PET images were spatially normalized to the Montreal neurological institute (MNI) space using SPM12 (fil.ion.ucl.ac.uk/spm/software/spm12) and intensity-normalized to obtain SUVR maps using a previously validated data-driven method based on histogram matching.[40] Region-of-interest (ROI) analysis was performed to calculate the average FDG uptake across all the VOIs defined in the Harvard-Oxford atlas. The cingulate island sign ratio (CISr), a well-established biomarker for distinguishing patients with DLB and AD,[41,42] was calculated as the ratio between the posterior cingulate cortex and the precuneus and cuneus uptake.[7,43] Further processing involved transforming each individual ROI value to z-scores (referenced to the healthy control group). Subsequently, individuals without notable hypometabolism (defined as $z \leq -1.5$) in any of the relevant brain areas associated with AD or DLB (Supplementary Table S1) were identified as Hypo-. Patients presenting significant hypometabolism in any AD or DLB related areas (Hypo+) where further classified as presenting AD-like or LB-like hypometabolism using a previously-developed classification algorithm based on autopsy-confirmed cases.[14]

Statistical analysis

Differences in demographics, neuropsychological variables and biomarkers were compared between groups using two-sample t-tests for normally distributed continuous variables (including the CISr), Mann-Whitney U tests for non-normally distributed and ordinal variables, and Fisher's exact tests for categorical variables (including clinical diagnostic outcomes, i.e., "dementia due to AD" vs "dementia due to other etiology").

Brain-wide hypometabolism patterns for each biomarker-defined group were assessed using voxel-wise two-sample t-tests of the SUVR maps between each group and the healthy control group using SPM12. Age and sex were used as confounding nuisance covariates.[44,45] Cohen's *d* values were derived from the t-values of the SPM maps as $d = t * \sqrt{\frac{1}{n1} + \frac{1}{n2}}$, where n1 and n2 correspond to the sample sizes of the two groups that are being compared.[46] We additionally calculated significance thresholds at $p<0.05$ (FDR) that were used for displaying the results. Spatial similarities between group-wise hypometabolism patterns were quantified using spatial

Spearman's correlation analysis of regional effect sizes across the 52 ROIs defined in the Harvard-Oxford atlas.[47] In complementary analyses, we compared the different biomarker-defined groups by focusing exclusively on Hypo+ individuals, which allows for a more direct comparison of the topography of the distinct hypometabolism patterns.

Differences in cognitive trajectories between the different groups were assessed using linear mixed effects models, which included patient-specific intercepts and slopes. Sex, age at baseline, and years of education were used as nuisance covariates for all models.

Finally, Cox proportional hazard models (lifelines.readthedocs.io) were used for assessing differential risks for developing hallucinations over disease progression between groups. Age, sex, global cognition (MMSE), and the presence/absence of hypometabolism (Hypo+/-) were used as covariates. For comparison, we also assessed differential risks for developing hallucinations as a function of the individual hypometabolism pattern classifications as AD- or LB-like using analogous Cox regression. The Breslow estimator was used for obtaining the regression parameters as well as the cumulative baseline hazard function.

Results

Baseline demographical and clinical data groups

335 patients (38.6%) were classified as AD+LB-, 158 (18.2%) were classified as AD+LB+, 68 (7.8%) were classified as AD-LB+, and 304 (35.0%) were classified as AD-LB-. Three patients were excluded due to presenting Type 2 αSyn aggregates. Table 1 summarizes demographical and neuropsychological data at baseline (for detailed pairwise comparisons between groups, see Supplementary Table S2). No differences in age were found between the pathological groups ($|d|<0.17$, $p<0.082$), but AD-LB- patients were significantly younger than any other group ($d<-0.24$, $p<0.039$). AD-LB+ patients were more likely to be male compared to all other groups (OR = [1.7 - 3.1], $p<0.051$). Regarding APOE ε4 positivity, no differences were observed between AD+LB- and AD+LB+ ($p=0.549$), while both AD-LB+ and AD-LB- were less frequently carriers of the ε4 alleles ($p<0.001$).

In neuropsychological assessments, all pathological groups were significantly more impaired than those in the AD-LB- group ($d>0.35$, $p<0.010$), and patients with mixed AD+LB+ pathology were more globally impaired than AD+LB- at baseline ($d=-0.27$, $p=0.005$), which was also reflected in a higher proportion of patients diagnosed with ADD (42% vs. 32%, $p=0.043$). However, according to the cognitive profile variables, both groups exhibited similar memory-predominant cognitive profiles (Δ(MEM-EF): $d=-0.12$, $p=0.213$; Δ(MEM-VS): $d=-0.17$, $p=0.075$). By contrast, patients in the AD-LB+ group had overall higher MMSE scores than the AD+ groups ($d>0.46$, $p<0.001$), and were particularly less impaired in the memory domain ($d>0.70$, $p<0.001$; executive function: $d>0.28$, $p<0.03$; visuospatial: $d>0.14$, $p>0.30$). Accordingly, AD-LB+ patients exhibited a primarily executive/visuospatial-predominant cognitive profile (Δ(MEM-EF)>0, $p=0.01$; Δ(MEM-VS)>0, $p=0.04$), which significantly differed from the AD+ groups ($d>0.40$, $p<0.003$).

Brain-wide hypometabolic patterns

Figure 1A presents the group-wise FDG-PET hypometabolism patterns for each of the biomarker-defined groups in comparison to the control group. As expected, the AD+LB- group, exhibited a classical AD-typical temporo-parietal pattern, including pronounced medial and lateral temporal effects extending to the lateral parietal cortex, posterior cingulate, and the precuneus, with a well-preserved occipital metabolism. Interestingly, the mixed AD+LB+ group was characterized by a nearly identical temporo-parietal hypometabolism pattern as the AD+LB- group, including the characteristic preservation of occipital metabolism, although the effect size of regional hypometabolism was notably higher in this group. By contrast, the AD-LB+ group showed a distinct DLB-typical pattern of pronounced posterior-occipital hypometabolism with a relative sparing of the medial temporal lobe and the posterior cingulate that was clearly differentiated from the AD+ groups. Consistently, ROI-based analyses (Figure 1B) fully reproduced and quantified the voxel-wise observations, revealing a significantly higher CISr in AD-LB+ compared to the AD+ groups ($d>0.53$, $p<0.001$), but no difference between the AD+LB- and the AD+LB+ groups ($d=0.04$, $p=0.607$). Finally, the AD-LB- group showed only very mild hypometabolism that was largely limited to the medial temporal lobe.

In complementary analyses aimed at comparing the regional hypometabolism patterns between groups more directly, we focused exclusively on individuals with evidence of any regional

hypometabolism (Hypo+), which represented 77%, 81%, 65%, and 53% of the AD+LB-, AD+LB+, AD-LB+, and AD-LB- groups, respectively. Voxel-wise analyses restricted to these Hypo+ individuals revealed largely identical but more pronounced group-wise patterns of hypometabolism (Supplementary Figure S1), including highly similar patterns of temporo-parietal hypometabolism in both AD+ groups (spatial correlation: ρ=0.96) and a clearly distinct pattern of posterior-occipital hypometabolism in AD-LB+ (spatial correlations: ρ<0.41). Pair-wise group comparisons further confirmed a significantly more severe occipital involvement in the AD-LB+ group compared to both AD+LB- and AD+LB+, and AD+LB+ patients also showed a slightly lower occipital metabolism compared to AD+LB- (d~0.2) in addition to more pronounced temporo-parietal involvement (Supplementary Figure S2). When classifying individual Hypo+ profiles into AD-like or LB-like patterns according to our pathology-informed classification algorithm, most of the patients in the AD+LB- (74.7%) and AD+LB+ (71.1%) groups were classified as showing an AD-typical hypometabolism pattern, whereas the majority of the AD-LB+ patients (70.4%) were classified as showing a LB-typical pattern.

Cognitive trajectories

Over longitudinal clinical follow-up (Figure 2), AD+LB+ patients showed a significantly faster global cognitive decline compared to those in the AD+LB- group (MMSE: d=-0.42, p<0.001), being faster progressors in the memory (ADNI-MEM: d=-0.37, p<0.001), executive function (ADNI-EF: d=-0.35, p<0.001), and visuospatial domains (ADNI-VS: d=0.19, p=0.06). However, both groups retained a similar memory-predominant cognitive profile over time (Δ(MEM-EXEC): d=0.05, p=0.53; Δ(MEM-VS): d=0.12, p=0.23).

By contrast, the AD-LB+ group showed a significantly slower global progression compared to the AD+ groups (d>0.77, p<0.001), which was mainly driven by a slower memory decline (d>0.61, p<0.001), wheras decline in the visuospatial domain was comparable to the AD+ groups (d<0.10, p>0.42). When compared to AD-LB-, the AD-LB+ group declined significantly faster in the executive function and visuospatial domains (d>-0.34, p<0.010), but not in memory function (p=0.13). Thus, the AD-LB+ group retained a distinguished executive/visuospatial-predominant cognitive profile over time.

Development of hallucinations

All the pathological groups had a significantly higher risk of developing hallucinations compared to the AD-LB- group (HR>16, p<0.001) (Figure 3, left). However, we did not observe an increased risk of developing hallucinations in the AD+LB+ group compared to the AD+LB- group (HR=1.28, CI = [0.72, 2.27], p=0.40). Interestingly, despite the considerably slower cognitive decline, the AD-LB+ group showed a similar risk for developing hallucinations as the AD+ groups (p>0.12). Regarding the model covariates, being female (HR=2.04, CI = [1.28, 3.33], p<0.001) and presenting any significant hypometabolism (Hypo+: HR=3.81, CI = [1.99, 7.29], p<0.001) were strong predictors for the future development of hallucinations, while global cognition (p=0.58) or age at baseline (p=0.08) were not. When stratifying patients according to the their type of hypometabolism pattern instead of biomarker profile (Hypo-, LB-like, AD-like), we observed that exhibiting a LB-like posterior-occipital pattern of hypometabolism was a strong predictor for the development of hallucinations, more than doubling the risk compared to an AD-like pattern (HR=2.58, CI = [1.60, 4.17], p=0.004). (Figure 3, right).

Changes in clinical diagnosis

Over clinical follow-up, 44% of the ADD patients in the AD-LB+ group changed their diagnosis from "probable AD" to "dementia due to other etiology", whereas this was only the case for 21% of the AD+ ADD patients (p=0.04), with no significant differences between the AD+LB- (26%) and AD+LB+ (17%) groups (p=0.19; Fig. 4A). Similarly, of the aMCI patients in the AD-LB+ group who converted to dementia over follow-up, 20% were diagnosed as "dementia due to other etiology", whereas this was only the case for 3% of the AD+ patients (p=0.06) (Fig. 4B). Moreover, among those AD-LB+ patients diagnosed as dementia due to AD at follow-up, a considerably higher proportion (23%) was diagnosed as "possible AD" (vs "probable AD") compared to the AD+LB- and AD+LB+ groups (5%, p=0.04), with no significant difference between the latter two (p=0.27).

Mixed AD+LB+ patients with AD-like vs LB-like hypometabolic patterns

Given that DLB is frequently underdiagnosed and that many DLB patients have AD co-pathology, in complementary analyses we wanted to further explore whether the smaller subset of patients in the mixed AD+LB+ group that were classified as having an LB-like hypometabolic pattern also showed other clinical features that would be consistent with DLB. At baseline, these LB-like AD+LB+ patients did not show any differences in demographic or cognitive variables compared to those with an AD-like pattern (Supplementary Table S3), and longitudinal decline in global and domain-specific cognitive scores was also comparable between these groups ($p>0.10$) (Supplementary Figure S3). However, LB-like AD+LB+ patients showed trends for being at a higher risk of developing hallucinations over follow-up (HR=1.52, p=0.07) and also to be more likely to evolve to a diagnosis of "dementia due to other etiologies" (38% vs 15%, p=0.09).

Discussion

In a large cohort of patients clinically diagnosed with aMCI or ADD, 57% of the patients showed a positive CSF-based biomarker profile of AD pathology (A+T+), of which 32% were also positive for LB pathology. In addition, a smaller but not negligible subset of 8% of the amnestic patients had biomarker evidence of LB pathology in the absence of a positive AD profile. These proportions were similar to those reported by previous studies using similar assays,[48] including those evaluating more diverse memory clinic cohorts including patients with clinical diagnoses of DLB or PD.[32] However, these numbers contrast with a reported neuropathological prevalence of LB co-pathology in AD of approximately 50%,[8] which may be due to the relatively low sensitivity of αSyn-SAAs for spatially restricted LB pathology. Thus, recent neuropathological validation studies have found that these assays mainly detect advanced neocortical LB pathology, with a lower sensitivity for limbic or amygdala-predominant LBs,[27] which are common in the setting of AD-LB co-pathology.[8,19,49]

With respect to the effects of LB co-pathology on the neurodegeneration pattern and clinical phenotype in the context of AD, AD+LB+ patients did not show FDG-PET characteristics associated with DLB, but rather a typical temporo-parietal hypometabolism that was largely

identical to that of AD+LB- subjects, including a relative sparing of the occipital lobe. Accordingly, the CISr, a well-established imaging biomarker aimed at distinguishing AD and DLB,[7,41,43] did not differ between AD patients with and without LB co-pathology. However, the AD+LB+ group did show higher effects sizes in AD-characteristic temporo-parietal areas, supporting the notion of a more severe disease course in AD patients with LB co-pathology.[17,23,24] Accordingly, the AD+LB+ group also showed a higher proportion of dementia patients (42% vs 32%) and more severe global cognitive impairment at baseline compared to the AD+LB- group. Interestingly, however, these differences were mainly driven by worse memory scores, and thus AD+LB+ patients did not show a differential cognitive profile, but rather an AD-typical memory-predominant cognitive profile comparable to the AD+LB- group. Longitudinally, the AD+LB+ patients progressed fastest in all cognitive domains, but they maintained the characteristic memory-predominant profile that did not differ from that of AD+LB- patients. Accordingly, both patient groups retained comparably high proportions of clinical diagnoses of "probable AD" over follow-up. Similarly, AD+LB+ patients did not show an elevated risk of developing hallucinations over follow-up compared to AD+LB- patients. Taken altogether, these results indicate that LB co-pathology in the context of AD (i.e., amnestic syndrome with AD+ biomarkers) is linked to a more aggressive disease course but does not generally associate with the development of a mixed clinical phenotype with DLB-like features. These in-vivo findings replicate and extend identical findings from our previous imaging-pathologic association study in a much smaller autopsy cohort where analogous AD/LB groups were defined based on neuropathologic information instead of CSF biomarkers[14]. However, we note that the clinical context may be key here, as a purely biomarker- (or pathologically-) defined AD+LB+ group not limited to amnestic syndromes may include a higher proportion of DLB cases with AD co-pathology (i.e., where LB pathology is the main pathologic driver of clinical symptoms), which may consequently affect the (average) clinical phenotype and disease trajectory of this group.[18,32]

In this context, it is interesting to note that although the AD+LB+ group clearly showed an AD-typical clinical phenotype and FDG-PET pattern when compared to healthy subjects, a direct voxel-wise comparison between the AD+LB+ and AD+LB- groups indicated slightly more pronounced (d~0.2) occipital lobe hypometabolism in the AD+LB+ group. These differences are likely driven by a small subset of AD+LB+ patients who were identified by our pattern classification analysis as showing an LB-like posterior-occipital FDG-PET pattern, rather than the AD-like pattern that

characterized most of the group. Interestingly, in contrast to the group's average clinical profile, these LB-like patients in the AD+LB+ group were indeed at a higher risk of developing hallucinations and they were also more likely to evolve to a diagnosis of "dementia due to other etiologies", although these differences only reached trend-level statistical significance in this relatively small subsample.

In addition to the comorbid AD+LB+ cases, we also identified a small subset (~8%) of amnestic patients who tested positive for LB but not for AD biomarkers (AD-LB+). This comparably small subset of amnestic patients with relatively pure LB pathology has been a consistent finding in neuropathological studies focusing on clinical AD,[11–15] but it has rarely been studied in detail. In contrast to the AD+LB+ group, this group did indeed show a distinct posterior-occipital hypometabolism pattern with a relative sparing of the medial temporal lobe that is typical for DLB (Figure 1, Suppl. Figs. 1 and 2). In addition, patients within this group also showed several other demographical and clinical features linked to LB pathology, including enrichment for male sex,[50] a lower frequency of APOE ε4 positivity,[51] and a more executive/visuospatial-predominant cognitive profile.[4] Compared to the AD+LB- and AD+LB+ groups, they were also significantly more likely to have their diagnosis changed to "dementia due to other etiology" or "possible AD" over follow-up. Altogether, these characteristics suggest that this group may be primarily composed of misdiagnosed (ADD) or prodromal (aMCI) DLB patients,[52,53] especially when taking into account that amnestic deficits are not rare in early stages of DLB[54,55]. While many of these patients may eventually meet full diagnostic criteria for DLB,[4] others may present mixed clinical profiles leading to less confidence in the clinical diagnosis of AD. Interestingly, these patients showed a relatively slow-paced cognitive decline compared to AD+, which mainly affected the executive function and visuospatial domains. Although DLB is typically described as showing a faster disease course compared to AD,[4,56,57] most of the studies comparing AD and DLB define their groups based on clinical diagnosis, and thus the DLB groups likely include a high proportion of cases with AD co-pathology.[58,59] Post-mortem studies have revealed that pure LB pathology associates with a milder clinical trajectory compared to these mixed cases,[60] which is in line with our current findings.

The lack of a significantly elevated risk for hallucinations in the LB+ groups (compared to AD+LB-) contrasts with our previous imaging study in which we found that amnestic patients with a LB-like posterior-occipital pattern of hypometabolism were more likely to develop hallucinations over

follow-up,[25] and we hypothesized that these subjects may represent a subset of amnestic patients in which LB (co-)pathology may have a stronger contribution to the observed neurodegeneration phenotype. Here we observed that neither the AD+LB+ nor the AD-LB+ groups were (group-wise) at a higher risk of developing hallucinations compared to the AD+LB- group. However, a similar risk of hallucinations in the AD-LB+ group despite a significantly slower cognitive decline compared to the AD+ patients indicates a relatively higher contribution of hallucinations to the clinical phenotype in this group. In line with that, we observed that presenting any hypometabolism (HR=3.81), and particularly a LB-like pattern (HR=2.58 vs AD-like), was highly associated with the development of hallucinations. While the overall proportion of individuals showing significant hypometabolism was lower in AD-LB+ compared to the AD+ groups, the large majority of those that presented regional hypometabolism showed an LB-typical pattern, and this pattern was also observed on an individual basis in 29% of the AD+LB+ group. However, unexpectedly the LB-like pattern was also observed in 25% of the AD+LB- group, indicating that a comparable posterior-occipital pattern of hypometabolism may also occur in the absence of LB pathology. While theoretically this group may also include cases with low-stage LB pathology which was not detectable by the αSyn-SAA,[27] it may also represent a subset of (relatively pure) AD patients exhibiting an atypical neurodegeneration phenotype involving the occipital lobe, such as reported for the rare posterior cortical atrophy (PCA) variant of AD.[61,62] Interestingly, in our previous imaging-neuropathological study we observed that it was not the presence of LBs by itself, but rather the presence of dopaminergic neurodegeneration (substantia nigra neuronal loss) which was associated with a more DLB-like hypometabolic pattern.[14] In this regard, it is worth mentioning that while far more prominent in LB diseases, dopaminergic degeneration is also present in AD, especially at advanced stages.[63–65] Future studies combining FDG-PET and αSyn-SAAs with imaging modalities aimed at evaluating dopaminergic neurodegeneration in-vivo [66–69] would be of great interest for a better understanding of this subgroup.

Our work also presents a series of limitations. First, our cohort is restricted to patients with typical AD-like clinical presentations, which limits the reach of our conclusions to this particular clinical setting. While we acknowledge that different effects of comorbid AD-LB pathology may be observed in clinically more diverse dementia cohorts,[32] predicting progression in clinical AD poses a distinct diagnostic challenge highly relevant for individual patient management. In this regard, our cohort provides unique insight into the role of αSyn-SAA and FDG-PET as complementary

tools for the phenotyping of amnestic patients.[8] Second, neuropsychological data collected within the ADNI study allows for the assessment of differential executive/visuospatial-predominant or amnestic-predominant neuropsychological profiles, but DLB core features are not systematically assessed in that study. While the NPIQ is a widely accepted instrument, more sophisticated metrics to assess the type and nature of hallucinations have been developed and could have provided more detailed insights into the differential risk for hallucinations associated with distinct AD/LB biomarker profiles and FDG-PET patterns.

Data availability

All patient data used in the preparation of this article are available through the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The processed datasets generated and/or analysed in the current study are available from the corresponding author upon reasonable request.

Acknowledgements

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. 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

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Hypometabolism patterns and CISr. A) Hypometabolism patterns of the biomarker-defined patient groups. Color represents effect size (Cohen's d) and white bars denote threshold for statistical significance at p(FDR)<0.05. B) CISr measurements of the biomarker-defined patient groups. The overlaid information provides Cohen's d and p-values for two-sample t-tests between the CISr values of the different biomarker-defined groups.

Figure 2 Global and domain-specific cognitive trajectories. Estimated cognitive trajectories of MMSE (top left), ADNI-MEM (top right), ADNI-EF (center left), ADNI-VS (center right), and cognitive profile variables Δ(MEM-EXEC) (bottom left) and Δ(MEM-VS) (bottom right) for each biomarker group. Group trajectories were estimated using covariate-adjusted linear mixed models with subject-specific intercepts.

Figure 3 Proportions of subjects remaining free of hallucinations. Predicted proportions of subjects remaining free of hallucinations according to the fitted Cox proportional hazard models for subjects within the different CSF-based biomarker groups (left) and subjects presenting the different hypometabolism patterns (right). Models are corrected for age, sex, and global cognition.

Figure 4 Diagnoses at the last available visit. Proportion of patients diagnosed as probable AD (dark orange), possible AD (light orange), and dementia due to other etiology (blue) at the last available visit, for each of the CSF-based biomarker groups. A) Subjects included as ADD ("probable AD") at baseline. B) aMCI to dementia converters.

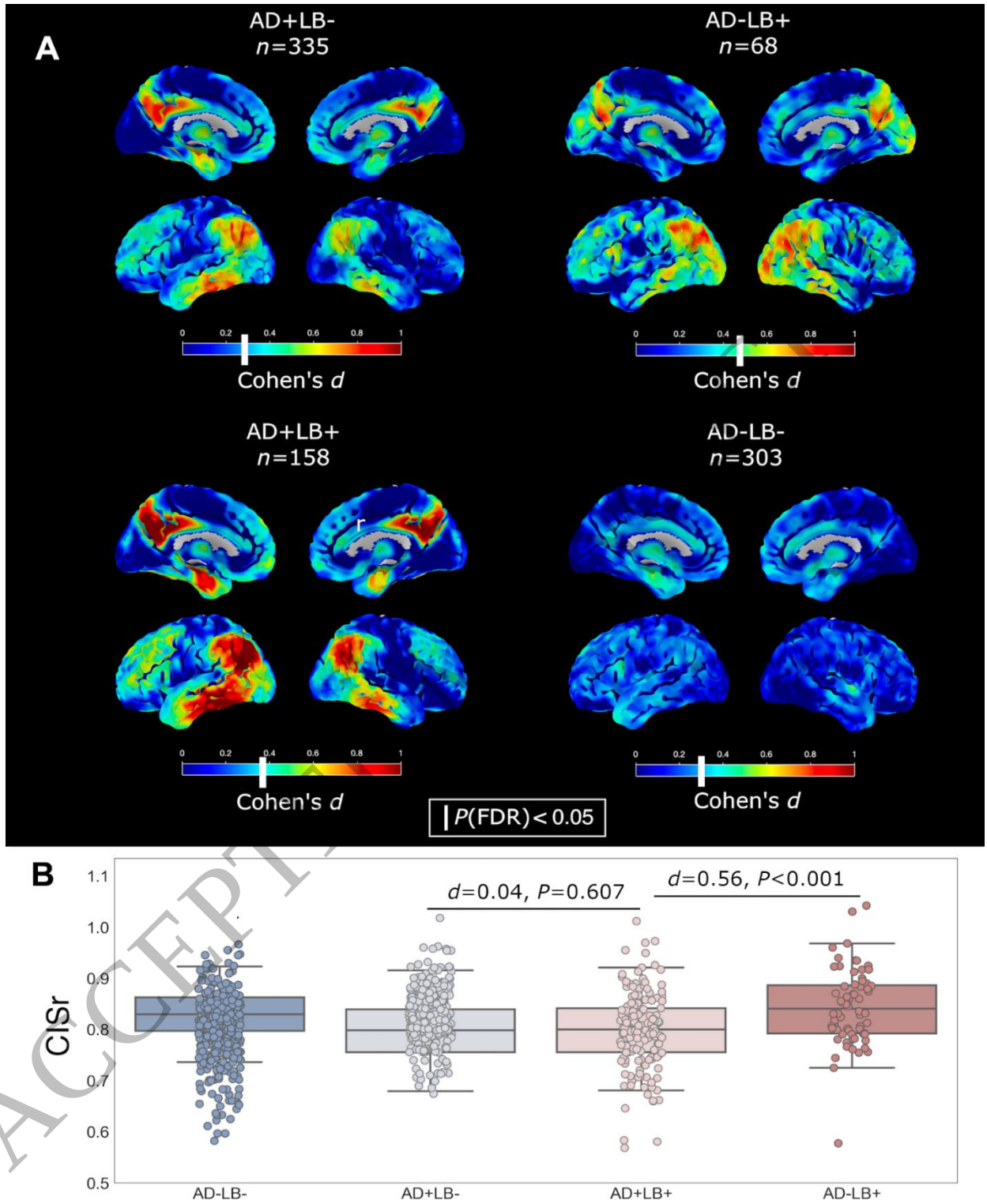

Figure 1
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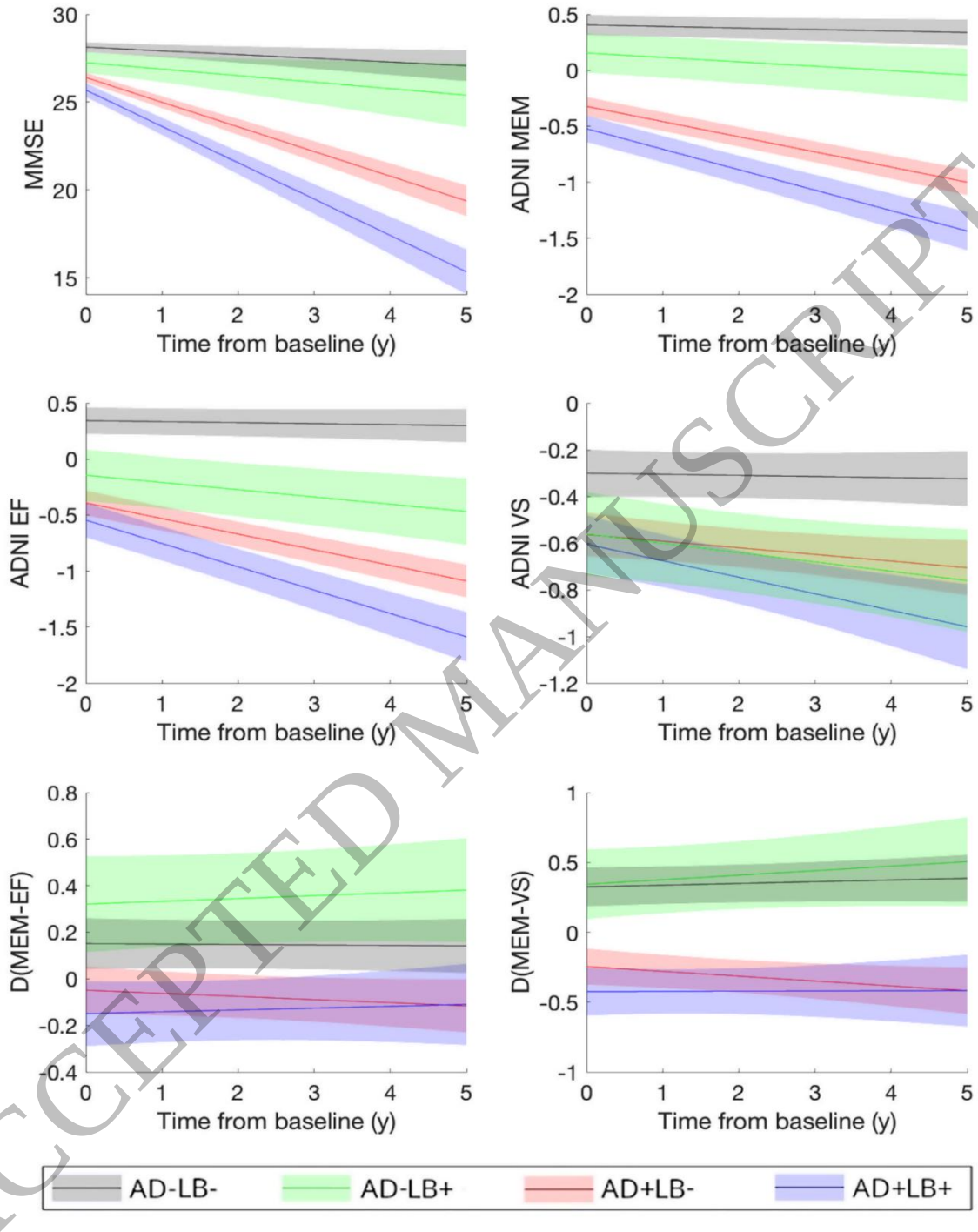

Figure 2
162x218 mm (x DPI)

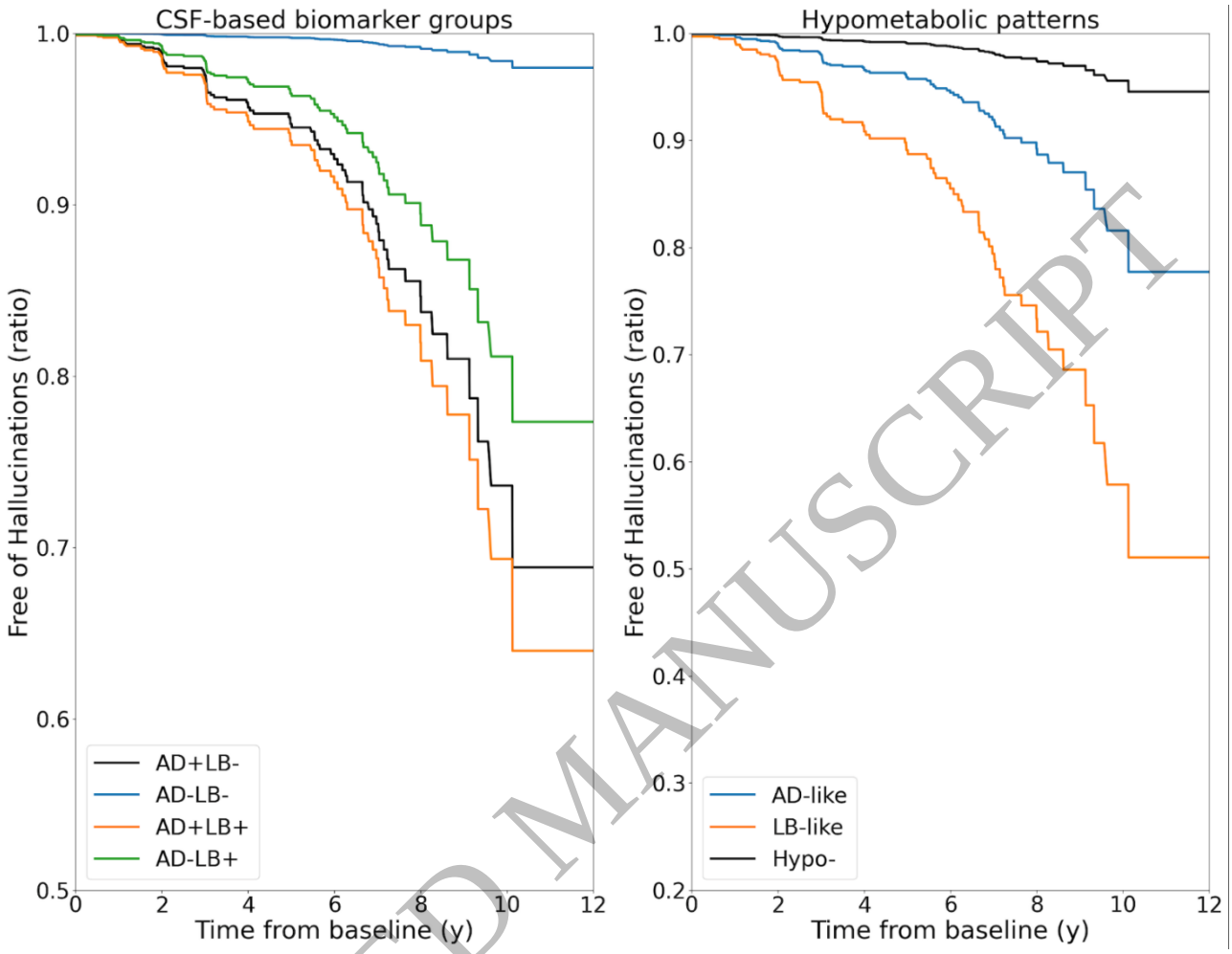

Figure 3
185x143 mm (x DPI)

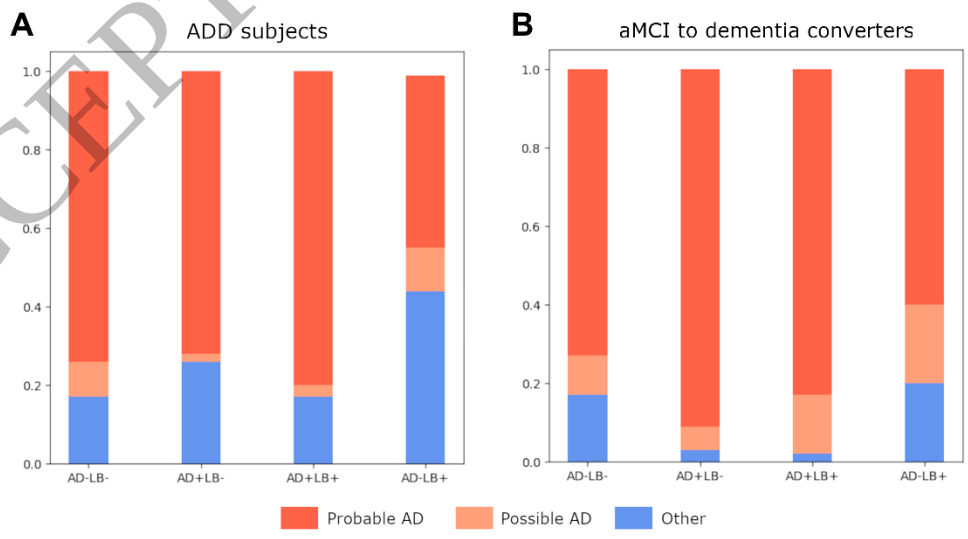

Figure 4
131x70 mm (x DPI)

Table 1 Demographical and clinical data for the different study groups

	AD-LB− (n=304)	AD+LB− (n=335)	AD+LB+ (n=158)	AD-LB+ (n=68)	One-way ANOVA*
Age, y	71.4 ± 8.4[a,b,c]	73.2 ± 7.2[d]	74.4 ± 7.6[d]	73.6 ± 7.7[d]	p=0.225
aMCI/ADD (%)	92/8[a,b]	68/32[b,c,d]	58/42[a,c,d]	87/13[a,b]	–
Male/Female (%)	58/42[c]	57/43[c]	59/41[c]	71/29[a,b,d]	–
APOE ε4, −−/+−/++, (%)	73/25/2[a,b]	28/51/21[c,d]	25/52/23[c,d]	73/24/3[a,b]	–
MMSE	28.1 ± 2.0[a,b,c]	26.2 ± 2.7[b,c,d]	25.4 ± 2.8[a,c,d]	27.3 ± 2.1[a,b,d]	p<0.001
ADNI-MEM	0.5 ± 0.7[a,b,c]	−0.3 ± 0.7[b,c,d]	−0.4 ± 0.7[a,c,d]	0.2 ± 0.7[a,b,d]	p<0.001
ADNI-EF	0.5 ± 0.8[a,b,c]	−0.2 ± 1.0[c,d]	−0.4 ± 0.9[c,d]	0.0 ± 1[a,b,d]	p=0.014
ADNI-VS	0.0 ± 0.7[a,b,c]	−0.3 ± 0.9[d]	−0.3 ± 0.8[d]	−0.2 ± 0.8[d]	p=0.471
Δ(MEM-EF)	0.1 ± 0.9[a,b,c]	−0.1 ± 0.8[d]	−0.2 ± 0.9[d]	0.3 ± 0.8[a,b,d]	p=0.002
Δ(MEM-VS)	0.4 ± 1.0[a,b]	−0.2 ± 1.1[c,d]	−0.4 ± 1.1[c,d]	0.3 ± 1.1[a,b]	p<0.001

MMSE = Mini-Mental State Examination; ADNI-MEM = ADNI Memory Composite Score; ADNI-EF = ADNI Executive Function Composite Score; ADNI-VS = ADNI Visuospatial Composite Score; Δ(MEM-EF) = Memory vs. executive function cognitive profile; Δ(MEM-VS) = Memory vs. visuospatial ability cognitive profile.

*P-values from ANOVA comparisons including the AD+LB−, AD−LB+ and AD+LB+ groups.

[a]Significantly different from AD+LB− at p<0.05 (two-sample t-test).

[b]Significantly different from AD+LB+ at p<0.05.

[c]Significantly different from AD−LB+ at p<0.05.

[d]Significantly different from AD−LB− at p<0.05