BMJ Open

The Association of Brain Amyloidosis with the incidence and frequency of Neuropsychiatric Symptoms in a Convenience Sample

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031947
Article Type:	Original research
Date Submitted by the Author:	27-May-2019
Complete List of Authors:	Goukasian, Naira; University of Vermont College of Medicine Hwang, Kristy; Emory University School of Medicine Grotts, Jonathan; University of California Los Angeles, Medicine Statistics Core Romero, Tamineh; University of California Los Angeles, Medicine Statistics Core Do, Triet; Tulane University School of Medicine Groh, Jenna; Indiana University School of Medicine, Neurology Bateman, Daniel; Indiana University School of Medicine, Psychiatry Apostolova, Liana; Indiana University School of Medicine, Neurology
Keywords:	Amyloidosis; Mild Cognitive Impairment (MCI), Alzheimer's disease (AD), Neuropsychiatric Symptoms (NPS), Alzheimer's Disease Neuroimaging Initiative (ADNI)

The Association of Brain Amyloidosis with the incidence and frequency of Neuropsychiatric Symptoms in a Convenience Sample

Naira Goukasian[1], Kristy S. Hwang[2], Jonathan Grotts[3], **Tamineh Romero[3],** Triet M. Do[4], **Jenna Groh [6]**, Daniel R. Bateman[5], Liana G. Apostolova [6,7,8] for the Alzheimer's Disease Neuroimaging Initiative*

* Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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 or in the provided supplementary ADNI Coinvestigator Appendix

[1] University of Vermont Larner College of Medicine, Burlington, VT, USA
[2] Emory University School of Medicine, Atlanta, GA, USA
[3] Department of Medicine Statistics Core, University of California, Los Angeles, CA, USA
[4] Tulane University Medical School, New Orleans, LA, USA
[5] Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA
[6] Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, USA
[7] Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, USA
[8] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

Submitted: 5/25/2019

Send correspondence to:
Liana Apostolova, MD, MSc, FAAN
Department of Neurology
Indiana University School of Medicine
355 W. 16th Street, Suite 4019
Indianapolis, IN 46202
Phone: +1 317/963-7436
Fax: +1 317/963-7533
E-mail: lapostol@iu.edu

Word count: Abstract 239, Body 3,798
Title: 122 characters (including spaces)
References: 43
Figures: 5
Tables: 1
Supplemental Tables: 3

KEYWORDS

Amyloidosis; Mild Cognitive Impairment (MCI); Alzheimer's disease (AD); Neuropsychiatric Symptoms (NPS); Alzheimer's Disease Neuroimaging Initiative (ADNI)

AUTHOR CONTRIBUTIONS

- Naira Goukasian assisted with data processing and analyses, and the completion of integral statistical analyses. Ms. Goukasian was the primary author responsible for drafting the manuscript.

- Kristy S. Hwang assisted with data processing and analyses, provided critical insights for interpretation of the results and participated in revising of the manuscript.

- Jonathan Grotts completed integral statistical analyses, provided critical insights for interpretation of our results and participated in revising of the manuscript

- Tamineh Romero completed integral statistical analyses, provided critical insights for interpretation of our results and participated in revising of the manuscript

- Triet M. Do completed some of the analyses and took part in revising of the manuscript.

- Daniel R. Bateman provided critical insights for interpretation of our results and participated in revising of the manuscript.

- Liana G. Apostolova was responsible for the study concept and design. She provided significant oversight over all analyses, interpretation of results, and participated in all stages of manuscript preparation.

FINANCIAL DISCLOSURES

- Naira Goukasian has no disclosures.

- Kristy S. Hwang has no disclosures.

- Jonathan Grotts has no disclosures

- Tamineh Romero has no disclosures

- Triet M. Do has no disclosures.

- Daniel R. Bateman has no disclosures.

- Liana G. Apostolova received research support from General Electric Healthcare, Piramal and Eli Lilly, served on the speaker's bureau for Eli Lilly & Company and Piramal Enterprises and on an advisory board for Eli Lilly & Company.

ACNOWLEDGEMENTS:

This work was generously supported by NIA R01 AG040770, NIA K02 AG048240, NIA P50 AG16570, NIA P30 AG010133, NIA U01 AG024904 and the Easton Consortium for Alzheimer's Drug Discovery and Biomarker Development.

Data used in this manuscript 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 only some participated in analysis or writing of this report (complete listing available at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf).

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack

Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

We would also like to acknowledge and thank Dr. David Elashoff from the Department of Medicine Statistics Core at the University of California, Los Angeles for his assistance with the statistical design of this manuscript.

ABSTRACT

Objective: To investigate the relationship between amyloid burden and frequency of existing and incidence of new neuropsychiatric symptoms (NPS) in elderly with and without cognitive decline.

Methods: 275 cognitively normal (NC), 100 subjective memory complaint (SMC), 559 MCI, and 143 AD dementia Alzheimer's Disease Neuroimaging Initiative subjects received [^{18}F]-Florbetapir PET scans. Yearly neuropsychiatric inventory (NPI) data were collected from the study partners at each visit. Mean standard uptake volume ratios (SUVR) normalized to whole cerebellum were obtained. **Positive amyloid PET scan was defined as mean SUVR ≥1.17.** Fisher-exact test was used to compare NPS frequency and incidence between amyloid positive and amyloid negative subjects. Survival analyses were used to estimate hazard ratios for developing the most common NPS by amyloid status.

Results: No differences in NPS frequency were seen between amyloid positive and amyloid negative NC, SMC, MCI, or dementia groups. MCI subjects with amyloid pathology however tended to have greater frequency*severity (FxS) of all NPS except for agitation, depression, nighttime disturbances and elation. MCI subjects with amyloid pathology were at greater risk for developing apathy, anxiety and agitation over time. Baseline presence of **agitation and apathy and new onset agitation, irritability and apathy** predicted faster conversion to dementia among MCI subjects.

Conclusions: Amyloid pathology is associated with greater rate of development of new NPS in MCI. Anxiety and delusions are significant predictors of amyloid pathology. Agitation, **irritability and apathy** are significant predictors for conversion from MCI to dementia.

ARTICLE SUMMARY

Strength and limitations of this study

- Our analyses used ADNI data; ADNI - the premier longitudinal biomarker study in AD, employs standardized subject assessment and MRI and PET scan collection protocols and meticulous data quality control across all study sites.
- A strength of our study is the relatively large sample size and the inclusion of all diagnostic groups across the AD spectrum including cognitively normal participants many of which are in the presymptomatic AD stages, as well as the the use of neuropsychiatric data collection tools that are administered to the caregivers and not the subject themselves.
- One of the limitations of this study is that the NPI and NPI-Q use structured questions focused on the frequency and severity of symptoms from the preceding month only. Therefore, intermittent NPS that were not manifested by the subjects in the pre-specified timeframe are not adequately captured.
- Another limitation to our study is that ADNI employs rigorous exclusion criteria typical of clinical trials and hence our study cohort might not be representative of the general population.
- **ADNI excludes subjects with preexisting depression (Geriatric depression scale score >5) which likewise could affect the generalization of the results.**

BACKGROUND

Alzheimer's disease (AD) is the most common form of dementia, accounting for 60-80% of all dementia cases. AD is characterized by cortical amyloid plaque and neurofibrillary tangle deposition, as well as progressive synaptic and neuronal loss. Recently developed amyloid positron emission tomography (PET) radioligands with high affinity for amyloid plaques, such as ^{18}F-Florbetaben, ^{18}F-Flutemetamol, and ^{18}F-Florbetapir, can provide reliable *in vivo* visualization of cortical fibrillary β-amyloid plaque deposition [1-3]. With amyloid accumulation beginning up to two decades prior to symptoms onset, these amyloid PET radiotracers can measure amyloid burden in the symptomatic, as well as the asymptomatic stages [4 5].

Neuropsychiatric symptoms (NPS) are prominent features of AD and mild cognitive impairment (MCI). 35-75% of MCI subjects experience at least one neuropsychiatric symptom with depression, apathy, and anxiety being the most prevalent [6-8]. In the dementia stage, apathy, agitation, and anxiety are most prevalent, followed by aberrant motor behavior, dysphoria and disinhibition [7 9].

NPS have been associated with higher likelihood for cognitive decline. Symptoms of depression, irritability, and agitation were found to predict cognitive decline among cognitively normal, MCI, as well as individuals with subjective cognitive concerns pooled together[10]. One population-based study found that the presence of symptoms such as agitation, apathy, anxiety, irritability, and depression in normal controls (NC) at baseline significantly predicted incident MCI [11]. Neuroticism and its underlying facets of anxiety, depression and stress were

associated with diagnosis of MCI and neurodegeneration [12]. Other studies have shown that the presence of one or more NPS in MCI, specifically symptoms of agitation, depression, and apathy, increase the risk of progression from MCI to AD dementia [10 13-19]. Most patients with AD experience at least one NPS during the course of disease progression [6]. NPS in dementia are associated with faster disease progression [20 21], increased mortality [15], and caregiver burden [22], as well as shorter time until nursing home admission [23 24].

To date a few groups have studied the associations between NPS and amyloid burden **and some have suggested that NPS reflect underlying amyloid pathology[25 26].** Studies have gone on to show that increased apathy was significantly associated with greater cortical amyloid burden in MCI [27]. Anxious MCI subjects were 3.1 times more likely to have abnormal cerebrospinal fluid amyloid β levels – a proxy marker for brain amyloidosis [28]. Another group found both anxiety and irritability to associate with greater amyloid burden across the AD spectrum [29]. Amyloid positive NC who reported anxiety experienced faster cognitive decline [30] and greater frequency of mood disturbances over the following year [31]. Taken together these data seem to suggest that at least some NPS are associated with amyloid pathology and cognitive decline.

Here we investigate the relationship between amyloid burden and incidence and frequency of NPS across the spectrum from normal cognition to dementia. We hypothesized that amyloid pathology will associate with higher incidence of NPS across all disease stages. We postulated that amyloid deposition will associate with apathy, anxiety, irritability and depression in the asymptomatic and early

symptomatic stages, and with psychosis, aberrant motor behaviors, disinhibition and agitation in the late symptomatic stages.

METHODS

Subjects:

Data used in these analyses were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD).

The first phase of ADNI, ADNI-1, recruited approximately 800 adults, ages 55 to 90 across 50 sites in the United States and Canada. The study sample consisted of approximately 200 older NC individuals, 400 people with late amnestic MCI (LMCI) and 200 people with mild AD. ADNI expanded enrollment criteria with the launch of ADNI-GO in 2009 and enrolled 200 additional subjects with early amnestic MCI (EMCI). ADNI-2 added approximately 650 newly enrolled subjects [150 NC, 100 subjects with subjective memory complaints (SMC), 100 EMCI, 150 LMCI and 150 mild AD].

The clinical description of the ADNI cohort has been previously published [32]. Diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders

Association (NINCDS-ADRDA) criteria [33]. AD subjects were required to have Mini Mental State Examination (MMSE) [34] scores between 20 and 26 and a Clinical Dementia Rating scale (CDR) [35] score of 0.5–1 at baseline. Qualifying MCI subjects had memory complaints, but no significant functional impairment, scored between 24 and 30 on the MMSE, had a global CDR score of 0.5, a CDR memory score of 0.5 or greater, and objective memory impairment on Wechsler Memory Scale – Logical Memory II test (WMS-LMII) [36]. NC and SMC subjects had MMSE scores between 24 and 30, a global CDR of 0 and did not meet criteria for MCI and AD. NC were devoid of cognitive concerns, while SMC subjects had significant memory concerns manifested in a score of ≥ 16 on the first 12 items of the Cognitive Change Index [37]. Subjects were excluded if they refused or were unable to undergo MRI, had other neurological disorders, active depression, or history of psychiatric diagnosis, alcohol, or substance dependence within the past 2 years, less than 6 years of education, or were not fluent in English or Spanish. Inclusion and diagnostic criteria, as well as procedures and protocols, for the ADNI studies can be found on http://www.adni-info.org/Scientists/ADNIStudyProcedures.html. Written informed consent was obtained from all participants. For more up-to-date information, see www.adni-info.org.

[^{18}F]-Florbetapir PET amyloid imaging was added in the ADNI-GO/2 stages of the study. We downloaded the clinical, behavioral and amyloid PET data of 275 NC, 100 SMC, 559 MCI and 143 AD subjects from the ADNI-1, ADNI-GO, and ADNI-2 databases on November 3, 2015. EMCI and LMCI were grouped in our analyses.

Neuropsychiatric Data:

Neuropsychiatric data were captured with the Neuropsychiatric Inventory (NPI) [38] and the NPI Questionnaire (NPI-Q) [39]. ADNI-1 used the NPI-Q while ADNI-GO/2 used the full version. Both versions assess twelve symptoms - delusions, hallucinations, agitation, anxiety, apathy, irritability, depression, euphoria, disinhibition, aberrant motor behavior, sleep and appetite. Interviewers ask structured questions about the presence and severity (as well as frequency in the NPI) of the symptoms in the past month to the study partner.

NPI or NPI-Q data from the baseline and all annual visits were obtained from LONI IDA on November 3, 2015. NPS at baseline were coded as “absent” if not endorsed and as “present” if endorsed by the study partner. In our longitudinal analyses, we coded symptoms in follow-up as “absent” if never endorsed by the study partner (including the baseline visit) and as “emerging de novo” if absent at baseline but reported at one or more follow-up visit. **Frequency x severity (FxS) scores were obtained for subjects that received the full NPI questionnaire at baseline.**

Imaging Data and Analysis:

A detailed description of AV-45 PET acquisition may be found at http://www.adni-info.org/Scientists/ADNIStudyProcedures.html. Briefly, 370 MBq (10 mCi +/- 10%) bolus injection of AV-45 was administered intravenously. Approximately 50 minutes after injection, a 20-minute continuous brain PET

imaging session collected a dynamic amyloid PET data consisting of four 5-minute frames. In our main analyses, we used the mean whole brain Standard Uptake Volume Ratios (SUVR) from University of California Berkeley (UCB) downloaded from ADNI's database on November 3, 2015. The mean whole brain SUVR was obtained by averaging the SUVR values across the frontal, anterior/posterior cingulate, lateral parietal and lateral temporal grey matter regions [40]. The UCB protocols for ^{18}F-Florbetapir preprocessing, co-registration and normalization have been previously described [40]. We defined a positive amyloid PET scan as mean SUVR ≥1.17 [41].

Statistical Analyses:

Demographic comparisons between amyloid positive and amyloid negative groups within diagnostic categories were done using Fisher-exact or Student's t-test statistics, as appropriate. Comparisons of the frequency of symptoms at baseline, as well as the emergence of new NPS in follow-up conditional on amyloid status were done using Fisher-exact test. **Using stepwise backwards logistical regression we also studied the predictive value of the presence/absence of NPI behaviors as well as their FxS scores on amyloid status while adjusting for age, sex, education and *APOE4*. All p-values were adjusted for multiple comparison correction using false discovery rate (FDR).**

Survival analyses using Cox proportional hazard regression models were used to determine 1) the hazard ratios for developing the five most common early NPI symptoms – apathy, anxiety, agitation, irritability, and depression – in amyloid

positive versus amyloid negative participants in the NC, subjective memory complaints (SMC) and MCI groups; 2) the hazard for conversion from MCI to dementia in all MCI subjects based on the presence of the top five most common early NPI symptoms at baseline; and 3) the hazard for conversion from MCI to dementia in MCI subjects who were free of the five most common NPI symptoms at baseline but developed them in follow-up. For analyses 2) and 3), subsequent visits were excluded once a subject was diagnosed with dementia. Subjects who reverted from MCI to NC (N=34) in follow-up were excluded from all analyses. We censored the data ignoring visits after dementia was diagnosed in our time to conversion Cox proportional hazard regression models. **All Cox proportional hazard regression models were corrected for age, education, and *APOE4* status. The Cox regression models were repeated while additionally controlling for amyloid status. P-values were corrected for multiple comparisons using FDR.**

All statistical tests were two-sided and a p-value below 0.05 was considered statistically significant. All statistical analyses were done in the R Statistical Computing Environment (R Core Team, Vienna, Austria).

RESULTS

Our sample consisted of a total of 1,077 ADNI subjects including 275 NC (26.5% amyloid positive), 100 SMC (28.0% amyloid positive), 559 MCI (52.0% amyloid positive), and 143 AD (82.5% amyloid positive) subjects with average

follow-up time of 3.2 ± 2.4 years (**Table**). Amyloid positive NC and SMC were significantly older and more likely to be Apolipoprotein E *ε4 (APOE4)* positive

Table: Demographic comparisons by amyloid status across the diagnostic groups

Variables	NC (N=275)			SMC (N=100)			MCI (N=559)			DEMENTIA (N=143)		
	Amyloid- (N=202)	Amyloid+ (N=73)	p-value	Amyloid- (N=72)	Amyloid+ (N=28)	p-value	Amyloid- (N=269)	Amyloid+ (N=290)	p-value	Amyloid- (N=25)	Amyloid+ (N=118)	p-value
Age, mean (SD)	73.3 (6.0)	76.1 (5.0)	**0.001**	71.5 (5.3)	74.6 (6.0)	**0.015**	70.7 (7.8)	73.1 (6.9)	**<0.001**	75.8 (7.2)	74.0 (8.3)	NS
Education, mean (SD)	16.7 (2.6)	15.9 (2.7)	**0.03**	16.9 (2.2)	16.4 (3.1)	NS	16.3 (2.6)	15.9 (2.8)	NS	16.4 (2.4)	15.7 (2.7)	NS
Gender, M/F %	52/48	41/59	NS	47/53	25/75	NS	56/44	58/42	NS	80/20	53/47	**0.015**
APOE ε4, 0/1/2 %	80/20/0	55/45/0	**<0.001**	81/19/0	46/54/0	**0.004**	76/24/0	29/68/3	**<0.001**	76/16/8	25/52/23	**<0.001**
MMSE, mean (SD)	29.0 (1.2)	29.2 (0.9)	NS	29.0 (1.3)	29.1 (0.8)	NS	28.4 (1.5)	27.6 (1.8)	**<0.001**	23.3 (2.4)	23.1 (2.1)	NS
CDR-SB, mean (SD)	0.03 (0.11)	0.04 (0.16)	NS	0.06 (0.16)	0.13 (0.22)	NS	1.3 (0.7)	1.6 (0.9)	**<0.001**	4.3 (1.8)	4.5 (1.7)	NS
ADAScog-11, mean (SD)	5.6 (3.0)	6.4 (3.0)	**0.048**	5.5 (2.8)	5.9 (2.6)	NS	8.0 (3.7)	10.5 (4.5)	**<0.001**	17.6 (6.2)	21.3 (7.4)	**0.019**
FAQ, mean (SD)	0.2 (0.7)	0.1 (0.5)	NS	0.6 (2.0)	0.5 (1.0)	NS	1.9 (3.2)	3.5 (4.2)	**<0.001**	12.3 (7.8)	13.2 (7.0)	NS
NPI total, mean (SD)	1.0 (2.5)	0.7 (1.2)	NS	1.8 (3.8)	1.1 (1.8)	NS	3.5 (6.0)	4.6 (6.7)	NS	10.0 (9.1)	7.3 (8.8)	NS
Years of follow-up, mean (SD)	4.0 (2.9)	4.3 (2.9)	NS	1.6 (0.8)	1.2 (1.0)	NS	3.6 (2.1)	3.5 (2.2)	NS	1.1 (0.9)	1.1 (0.7)	NS

relative to their amyloid negative counterparts. Amyloid positive NC were also less educated and performed worse on the Alzheimer's Disease Assessment Scale – Cognition Subscale 11 (ADAScog-11) compared to amyloid negative NC. Amyloid positive MCI were significantly older, more likely to be *APOE4* positive, and showed greater impairment on Mini-Mental State Examination (MMSE), Clinical Dementia Rating – Sum of Boxes (CDR-SB), ADAScog-11, and Functional Assessment Questionnaire (FAQ) scales compared to amyloid negative MCI. Amyloid positive dementia subjects were more likely to be *APOE4* positive, and showed greater impairment on the ADAScog-11 compared to the amyloid negative dementia subgroup. No significant difference in years of follow-up was seen

between the amyloid positive vs. negative subjects in each of the diagnostic groups (**Table**).

Neuropsychiatric Symptoms at baseline:

No differences in NPS frequency at baseline were seen between amyloid positive and amyloid negative NC, SMC, MCI, or dementia groups (**Figure 1** and **Supplementary Table 1**). **Comparing NPI FxS means between the amyloid positive and negative subgroups within each diagnostic group revealed significantly greater aberrant motor behaviors FxS in amyloid positive vs. amyloid negative NC (0.083 vs. 0.005, p=0.04). Compared to amyloid negative MCI, amyloid positive MCI manifested significantly greater anxiety FxS (0.569 vs. 0.314, p=0.023), delusions FxS (0.138 vs. 0.004, p=0.001), and aberrant motor behaviors FxS (0.286 vs. 0.088, p=0.025) and trending appetite changes FxS (0.512 vs. 0.291, p=0.084). Compared to amyloid negative dementia subjects, amyloid positive dementia cases showed significantly lower apathy FxS (1.271 vs. 2.583, p=0.011), agitation FxS (0.619 vs. 1.708, p=0.003) and appetite changes (0.864 vs. 2.417, p=0.006), however these findings should be cautiously interpreted due to the very small sample size of the amyloid negative dementia group (N=25 vs. N=118 amyloid positive). No significant differences were seen in SMC. The full statistical models can be seen in Supplementary Table 2.**

Cumulative incidence of NPS in follow-up:

No differences in the rates of de novo development of NPS were seen between amyloid positive and amyloid negative subjects in the NC, SMC or dementia groups. New onset delusions (13.4% vs. 2.2%, p<0.001), hallucinations (8.0% vs. 2.2%, p=0.007), anxiety (35.9% vs. 24.8%, p=0.014), apathy (38.4% vs. 21.8%, p<0.001), disinhibition (24.2% vs. 14.7%, p=0.014), irritability (45.5% vs. 32.7%, p=0.014), aberrant motor disturbances (18.1% vs. 9.2%, p=0.008**), and appetite disturbances (33.5% vs. 20.9%, p=0.007)** were significantly more common in amyloid positive vs. amyloid negative MCI (**Figure 2**). **The full statistical models can be seen in Supplemental Table 3.**

Predictors of amyloid status at baseline:

In our pooled stepwise backwards logistic regression model, anxiety [odds ratio (OR)=1.87, 95% confidence interval (95%CI) 1.17-3.02, p=0.018] and delusions (OR=7.01, 95%CI 1.25-132.05, p=0.04) were predictive of amyloid positivity after correcting for age, sex, education, and *APOE4* in the pooled sample. When we analyzed the same relationship using the continuous FxS measure, delusions were a significant predictor (OR=2.22, 95%CI 1.18-6.23, p=0.012) while anxiety (OR=1.13, 95%CI 0.99-1.3, p=0.062) and aberrant motor behaviors (OR=1.22, 95%CI 0.99-1.56, p=0.062) were trending.

Among MCI participants the presence of anxiety (OR=1.77, 95%CI 1.03-3.09, p=0.04) at baseline was predictive of AD pathology. Using the continuous FxS measure revealed significant association between delusions

and amyloid pathology (OR=5.8, 95%CI 1.69-98.36, p=0.001), while anxiety was trending (OR=1.16, 95%CI 1.0-1.37, p=0.056).

No significant differences were seen among the individual NC, SMC, or dementia groups.

Time to development of the five most frequent early NPS by amyloid status (Figure 3):

Here we focused on the five most common early NPS – apathy, anxiety, agitation, irritability and depression. Time to de novo development of these NPS in NC and SMC did not differ by amyloid status. **After controlling for age, education, and *APOE4* status, amyloid pathology in MCI was associated with faster emergence of agitation [hazard ratio (HR)=1.47, 95%CI 1.09-1.99, p=0.012], anxiety (HR=1.45, 95%CI 1.07-1.98, p=0.017), and apathy (HR=1.34, 95%CI 0.98-1.83, p<0.001).**

Effect of baseline NPS on time to conversion in MCI (Figure 4):

Individually, controlling for age, education and *APOE4* status, four of the five early NPS at baseline **were** significantly associated with time to conversion from MCI to dementia (agitation HR=2**.28,** 95%CI 1.**64-**3.**17,** p<0.001; depression HR=1.**51,** 95%CI 1.**08-**2.**11,** p=0.0**21;** irritability HR=1.**88,** 95%CI 1.**35-**2.**63, p**<0.0**01; anxiety HR=1.39, 95%CI 0.96-2.02, p=0.083;** apathy HR=**2.69,** 95%CI **1.92-3.78,** p<0.001**). Additionally, correcting for amyloid status did not change the results (agitation HR=1.78, 95%CI 1.27-2.49, p=0.002**; **depression**

HR=1.44, 95%CI 1.03-2.02, p=0.04; irritability HR=1.61, 95%CI 1.15-2.25, p=0.009; anxiety HR=1.2, 95%CI 0.82-1.75, p=0.341; apathy HR=2.57, 95%CI 1.83-3.61, p<0.001).

When all five symptoms were entered simultaneously into a multivariable Cox regression model **controlling for age, education and *APOE4* status agitation and apathy** remained significant (agitation HR=**1.76**, 95%CI **1.19-2.6**, p=0.00**5**; **apathy HR=2.59**, **95%CI 1.81-3.71**, **p<0.001**). **After additionally controlling for baseline amyloid status apathy remained significant (agitation HR=1.46, 95%CI 0.99-2.15, p=0.057**; **apathy HR=2.48, 95%CI 1.73-3.56, p<0.001).**

Effect of de novo NPS on time to conversion in MCI (Figure 5):

Individually, controlling for age, education and *APOE4* status, the emergence of agitation, **depression, anxiety** and apathy were associated with greater risk for conversion from MCI to dementia (agitation HR=**2.17,** 95%CI 1**.47-3.2,** p**<**0.00**1**; **depression HR=1.54, 95%CI 1.02-2.33, p=0.049; anxiety HR=2.17, 95%CI 1.47-3.2, p<0.001;** apathy HR=**2.33, 95%CI 1.57-3.45**, p<0.001**). Agitation, anxiety, and apathy remained significant when we also corrected for amyloid status (agitation HR=2.03, 95%CI 1.37-3, p=0.001**; **anxiety HR=2.03, 95%CI 1.37-3, p=0.001; apathy HR=2.42, 95%CI 1.65-3.57, p<0.001).**

When all five symptoms were entered simultaneously into a multivariable Cox regression model **controlling for age, education and *APOE4* status, de novo** agitation, **irritability, and apathy** were significant predictors of conversion

from MCI to dementia (agitation HR=**2.0,** 95%CI 1.**17-3.43**, p**=**0.0**12; irritability HR=0.45, 95%CI 0.24-0.82, p=0.009; apathy HR=2.09, 95%CI 1.17-3.73, p=0.012**). **Additionally, controlling for baseline amyloid status, did not change the results** (**agitation HR=1.91, 95%CI 1.12-3.28, p=0.018; irritability HR=0.47, 95%CI 0.26-0.86, p=0.014; apathy HR=2.42, 95%CI 1.39-4.23, p=0.002**).

DISCUSSION

Here we investigated the relationship between amyloid burden and incidence and frequency of NPS across the spectrum from normal cognition to dementia. We hypothesized that amyloid pathology will associate with higher frequency of NPS across all disease stages, but we found that **while the frequency of NPS is the not significantly different between amyloid negative and positive diagnostic groups, some NPS might be more severe in those who are amyloid positive (see FxS results and Supplementary Table 2). This held true for aberrant motor behaviors among amyloid positive NC and MCI, as well as anxiety and delusions in amyloid positive MCI. Our findings in the MCI group is in line with those by Krell-Roesch et al., 2019, who found that compared to MCI without amyloid burden, MCI with amyloid burden was associated with an increased risk of having NPS though they did not find this to be true in NC with amyloid burden[26]. Looking into whether neuropsychiatric symptoms carry prognostic value of Alzheimer's pathology**

we found anxiety to be predictive of brain amyloidosis in the pooled and MCI samples. Delusions carried additional predictive value in MCI.

In line with our hypothesis, a greater proportion of the amyloid positive MCI had emergence of delusions, hallucinations, anxiety, apathy, disinhibition, irritability, aberrant motor behaviors and appetite changes compared to amyloid negative MCI over the course of the study (**Figure 2)**. These findings are as expected considering the prominence and progressive development of NPS over time during the course of the disease [6 7]. Our Cox proportional hazard regression model further demonstrated that in the MCI due to AD develop apathy, anxiety, and agitation - three of the earliest and most pervasive NPS in the MCI stage, earlier compered to amyloid negative MCI (**Figure 3)** [6].

The survival analyses looking into the effects of the five most frequent early NPS on time to conversion from MCI to dementia showed that **four of the 5 early NPS** symptoms **– agitation, apathy, depression and irritability**, were individually predictive of conversion from MCI to dementia as previously reported [10 13-19]. When all five NPS were entered in a single predictive model **agitation and apathy** remained significant [13 14 17].

When we focused on the predictive effects of newly emerging symptoms on future conversion from MCI to dementia, **4 of the 5 symptoms** - **agitation, depression, anxiety and apathy,** were individually predictive. **When all five NPS were entered in a single predictive model agitation, irritability, and apathy proved to be significant.**

Taken together our data seem to indicate that both prevalent **agitation, and apathy,** and incident **agitation, irritability and apathy** are predictive of faster functional decline and loss of independence among amyloid positive and negative MCI. Our findings regarding agitation seem to agree with those reported by Brodaty et al.[13] who also found that agitation was significantly associated with cognitive decline. **Several studies have also concluded that apathy is a useful NPS for identifying cognitive decline to AD[18 42]. Affective symptoms, including irritability, have also been previously reported in association with later cognitive decline to dementia.** [43]

Contrary to our expectations and the observations of others [11 30] NPS were not associated with faster cognitive decline among ADNI's amyloid positive NC. We could hypothesize that both the sample size differences (N=275 in our study vs. N=1,587 in the large population-based study by Geda et al.[11]) as well as the different inclusion criteria used by these two studies (i.e., the mandatory lack of even perceived age-associated decline **and the exclusion of individuals with baseline depression defined as GDS>5** in ADNI NC) **and the fact that Geda et al. did not have information regarding brain amyloidosis,** as potential drivers for these differences.

Several strengths and limitations of the ADNI study should be acknowledged. One of the strengths of this study is our cohort. ADNI is the premier longitudinal biomarker study in AD. ADNI employs unified subject assessment and PET scan collection protocols and meticulous data quality control across all study sites. Another strength of our study is the relatively large sample size and the

inclusion of all diagnostic groups across the AD spectrum instead of just the symptomatic ones as it has been done in the past. Another strength of our paper is the use of neuropsychiatric data collection tools that are administered to the caregivers and not the subject themselves. One of the limitations of this study is that the NPI and NPI-Q use structured questions focused on the presence and severity of symptoms from the preceding month only, and therefore, intermittent NPS that were not manifested by the subjects in the pre-specified timeframe were not captured. Another limitation to our study is that ADNI employs rigorous exclusion criteria typical of clinical trials and the study population and hence our study cohort might not be representative of the general population. **Moreover, ADNI criteria excludes subjects with GDS greater than or equal to 5 and any primary psychiatric condition. These selection criteria undoubtedly have further influenced on our study and might limit the overall generalizability of our findings to the elderly population as a whole.**

In summary, we investigated the relationship between amyloid burden and occurrence of NPS in elderly with and without cognitive decline at baseline and over time. We found that amyloid pathology is a significant risk factor for future development of NPS in MCI, but not in the presymptomatic or at-risk stages of the disease. **We also found that the presence of apathy, agitation, depression and irritability in MCI patients predict a more aggressive disease course regardless of the presence or absence of amyloid pathology.**

References

1. Barthel H, Gertz HJ, Dresel S, et al. Cerebral amyloid-beta PET with florbetaben (18F) in patients with Alzheimer's disease and healthy controls: a multicentre phase 2 diagnostic study. *Lancet Neurol* 2011;10(5):424-35. doi: 10.1016/S1474-4422(11)70077-1
2. Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA* 2011;305(3):275-83. doi: 10.1001/jama.2010.2008
3. Vandenberghe R, Van Laere K, Ivanoiu A, et al. 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: a phase 2 trial. *Ann Neurol* 2010;68(3):319-29. doi: 10.1002/ana.22068
4. Dubois B, Hampel H, Feldman HH, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement* 2016;12(3):292-323. doi: 10.1016/j.jalz.2016.02.002
5. Sperling R, Mormino E, Johnson K. The evolution of preclinical Alzheimer's disease: implications for prevention trials. *Neuron* 2014;84(3):608-22. doi: 10.1016/j.neuron.2014.10.038
6. Apostolova LG, Cummings JL. Neuropsychiatric manifestations in mild cognitive impairment: a systematic review of the literature. *Dement Geriatr Cogn Disord* 2008;25(2):115-26. doi: 000112509 [pii]
10.1159/000112509 [published Online First: 2007/12/19]
7. Mega MS, Cummings JL, Fiorello T, et al. The spectrum of behavioral changes in Alzheimer's disease. *Neurology* 1996;46(1):130-5.
8. Steinberg M, Shao H, Zandi P, et al. Point and 5-year period prevalence of neuropsychiatric symptoms in dementia: the Cache County Study. *Int J Geriatr Psychiatry* 2008;23(2):170-7. doi: 10.1002/gps.1858 [published Online First: 2007/07/04]
9. Lyketsos CG, Steinberg M, Tschanz JT, et al. Mental and behavioral disturbances in dementia: findings from the Cache County Study on Memory in Aging. *Am J Psychiatry* 2000;157(5):708-14.
10. Donovan NJ, Amariglio RE, Zoller AS, et al. Subjective cognitive concerns and neuropsychiatric predictors of progression to the early clinical stages of Alzheimer disease. *Am J Geriatr Psychiatry* 2014;22(12):1642-51. doi: 10.1016/j.jagp.2014.02.007
11. Geda YE, Roberts RO, Mielke MM, et al. Baseline neuropsychiatric symptoms and the risk of incident mild cognitive impairment: a population-based study. *Am J Psychiatry* 2014;171(5):572-81. doi: 10.1176/appi.ajp.2014.13060821
12. Zufferey V, Donati A, Popp J, et al. Neuroticism, depression, and anxiety traits exacerbate the state of cognitive impairment and hippocampal vulnerability to Alzheimer's disease. *Alzheimers Dement (Amst)* 2017;7:107-14. doi: 10.1016/j.dadm.2017.05.002 [published Online First: 2017/06/28]
13. Brodaty H, Heffernan M, Draper B, et al. Neuropsychiatric symptoms in older people with and without cognitive impairment. *J Alzheimers Dis* 2012;31(2):411-20. doi: 10.3233/JAD-2012-120169

14. Palmer K, Di Iulio F, Varsi AE, et al. Neuropsychiatric predictors of progression from amnestic-mild cognitive impairment to Alzheimer's disease: the role of depression and apathy. *J Alzheimers Dis* 2010;20(1):175-83. doi: 10.3233/JAD-2010-1352
15. Pink A, Stokin GB, Bartley MM, et al. Neuropsychiatric symptoms, APOE epsilon4, and the risk of incident dementia: a population-based study. *Neurology* 2015;84(9):935-43. doi: 10.1212/WNL.0000000000001307
16. Rosenberg PB, Mielke MM, Appleby BS, et al. The association of neuropsychiatric symptoms in MCI with incident dementia and Alzheimer disease. *Am J Geriatr Psychiatry* 2013;21(7):685-95. doi: 10.1016/j.jagp.2013.01.006
17. Somme J, Fernandez-Martinez M, Molano A, et al. Neuropsychiatric symptoms in amnestic mild cognitive impairment: increased risk and faster progression to dementia. *Curr Alzheimer Res* 2013;10(1):86-94.
18. Teng E, Lu PH, Cummings JL. Neuropsychiatric symptoms are associated with progression from mild cognitive impairment to Alzheimer's disease. *Dement Geriatr Cogn Disord* 2007;24(4):253-9. doi: 10.1159/000107100
19. Wadsworth LP, Lorius N, Donovan NJ, et al. Neuropsychiatric symptoms and global functional impairment along the Alzheimer's continuum. *Dement Geriatr Cogn Disord* 2012;34(2):96-111. doi: 000342119
20. Peters ME, Schwartz S, Han D, et al. Neuropsychiatric symptoms as predictors of progression to severe Alzheimer's dementia and death: the Cache County Dementia Progression Study. *Am J Psychiatry* 2015;172(5):460-5. doi: 10.1176/appi.ajp.2014.14040480 [published Online First: 2015/01/15]
21. Rabins PV, Schwartz S, Black BS, et al. Predictors of progression to severe Alzheimer's disease in an incidence sample. *Alzheimers Dement* 2013;9(2):204-7. doi: 10.1016/j.jalz.2012.01.003 [published Online First: 2012/11/06]
22. Fischer CE, Ismail Z, Schweizer TA. Impact of neuropsychiatric symptoms on caregiver burden in patients with Alzheimer's disease. *Neurodegenerative Disease Management* 2012;2:269-77.
23. Cepoiu-Martin M, Tam-Tham H, Patten S, et al. Predictors of long-term care placement in persons with dementia: a systematic review and meta-analysis. *Int J Geriatr Psychiatry* 2016;31(11):1151-71. doi: 10.1002/gps.4449 [published Online First: 2016/10/19]
24. Gaugler JE, Yu F, Krichbaum K, et al. Predictors of nursing home admission for persons with dementia. *Med Care* 2009;47(2):191-8. doi: 10.1097/MLR.0b013e31818457ce [published Online First: 2009/01/27]
25. Geda YE, Schneider LS, Gitlin LN, et al. Neuropsychiatric symptoms in Alzheimer's disease: past progress and anticipation of the future. *Alzheimers Dement* 2013;9(5):602-8. doi: 10.1016/j.jalz.2012.12.001 [published Online First: 2013/04/09]
26. Krell-Roesch J, Vassilaki M, Mielke MM, et al. Cortical beta-amyloid burden, neuropsychiatric symptoms, and cognitive status: the Mayo Clinic Study of Aging. *Transl Psychiatry* 2019;9(1):123. doi: 10.1038/s41398-019-0456-z [published Online First: 2019/03/30]

27. Marshall GA, Donovan NJ, Lorius N, et al. Apathy is associated with increased amyloid burden in mild cognitive impairment. *J Neuropsychiatry Clin Neurosci* 2013;25(4):302-7. doi: 10.1176/appi.neuropsych.12060156
28. Ramakers IH, Verhey FR, Scheltens P, et al. Anxiety is related to Alzheimer cerebrospinal fluid markers in subjects with mild cognitive impairment. *Psychol Med* 2013;43(5):911-20. doi: 10.1017/S0033291712001870
29. Bensamoun D, Guignard R, Furst AJ, et al. Associations between Neuropsychiatric Symptoms and Cerebral Amyloid Deposition in Cognitively Impaired Elderly People. *J Alzheimers Dis* 2016;49(2):387-98. doi: 10.3233/JAD-150181
30. Pietrzak RH, Lim YY, Neumeister A, et al. Amyloid-beta, anxiety, and cognitive decline in preclinical Alzheimer disease: a multicenter, prospective cohort study. *JAMA Psychiatry* 2015;72(3):284-91. doi: 10.1001/jamapsychiatry.2014.2476
31. Babulal GM, Ghoshal N, Head D, et al. Mood Changes in Cognitively Normal Older Adults are Linked to Alzheimer Disease Biomarker Levels. *Am J Geriatr Psychiatry* 2016;24(11):1095-104. doi: 10.1016/j.jagp.2016.04.004
32. Petersen RC, Aisen PS, Beckett LA, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. *Neurology* 2010;74(3):201-9. doi: 10.1212/WNL.0b013e3181cb3e25
33. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34(7):939-44.
34. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12(3):189-98.
35. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 1993;43(11):2412-4.
36. Wechsler D. WMS-R: Wechsler Memory Scale-Revised manual. San Antonio, TX: Psychological Corp., Harcourt Brace Jovanovich 1987.
37. Saykin AJ, Wishart HA, Rabin LA, et al. Older adults with cognitive complaints show brain atrophy similar to that of amnestic MCI. *Neurology* 2006;67(5):834-42. doi: 10.1212/01.wnl.0000234032.77541.a2
38. Cummings JL. The Neuropsychiatric Inventory: assessing psychopathology in dementia patients. *Neurology* 1997;48(5 Suppl 6):S10-6.
39. Kaufer DI, Cummings JL, Ketchel P, et al. Validation of the NPI-Q, a brief clinical form of the Neuropsychiatric Inventory. *J Neuropsychiatry Clin Neurosci* 2000;12(2):233-9. [published Online First: 2000/09/23]
40. Jagust WJ, Bandy D, Chen K, et al. The Alzheimer's Disease Neuroimaging Initiative positron emission tomography core. *Alzheimers Dement* 2010;6(3):221-9. doi: 10.1016/j.jalz.2010.03.003
41. Fleisher AS, Chen K, Liu X, et al. Using positron emission tomography and florbetapir F18 to image cortical amyloid in patients with mild cognitive impairment or dementia due to Alzheimer disease. *Arch Neurol* 2011;68(11):1404-11. doi: 10.1001/archneurol.2011.150

42. Starkstein SE, Jorge R, Mizrahi R, et al. A prospective longitudinal study of apathy in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2006;77(1):8-11. doi: 10.1136/jnnp.2005.069575 [published Online First: 2005/12/20]
43. Ismail Z, Gatchel J, Bateman DR, et al. Affective and emotional dysregulation as pre-dementia risk markers: exploring the mild behavioral impairment symptoms of depression, anxiety, irritability, and euphoria - CORRIGENDUM. *Int Psychogeriatr* 2019;31(1):157. doi: 10.1017/S1041610218000637 [published Online First: 2018/11/22]

FIGURE LEGENDS

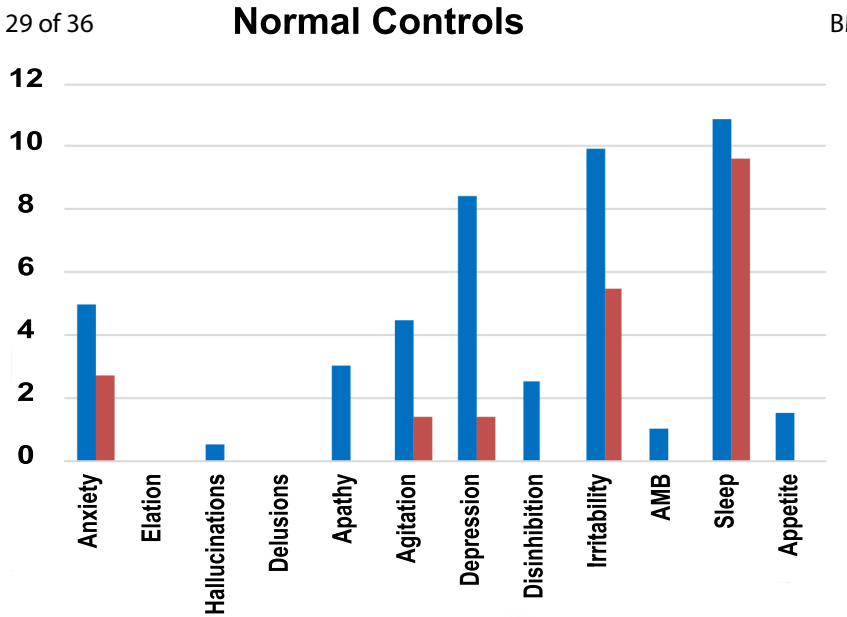
Figure 1: Baseline frequency of NPS by diagnosis and amyloid status. **NOTE: y-axis vary by diagnosis**

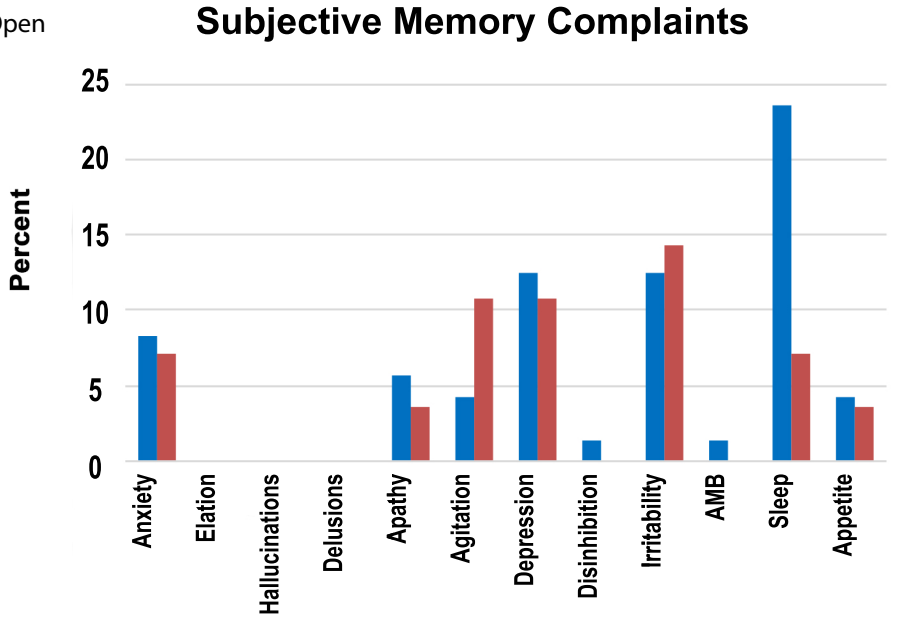
Figure 2: De novo emergence of NPS by diagnosis and amyloid status. NOTE: y-axis vary by diagnosis

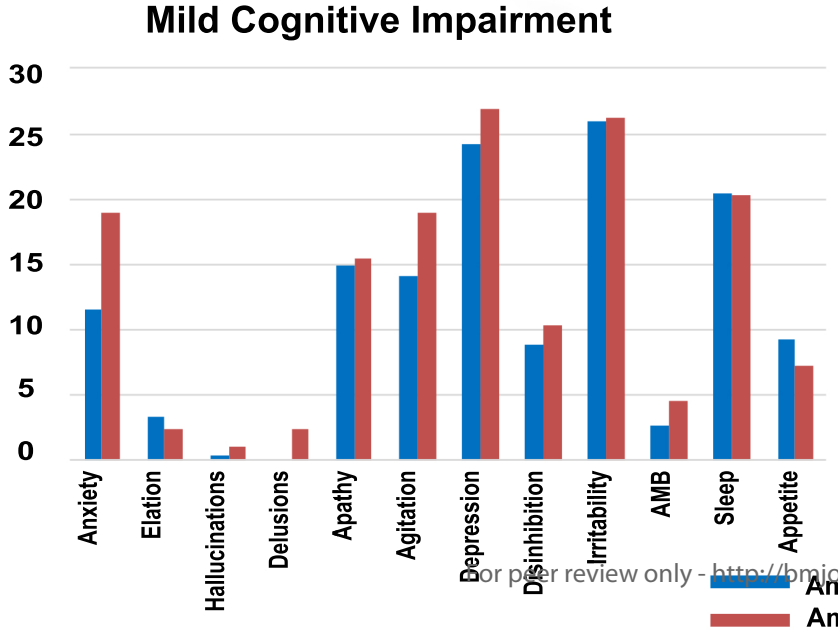
Figure 3: Survival curves showing time to emergence of the five most frequent early NPS in MCI by amyloid status

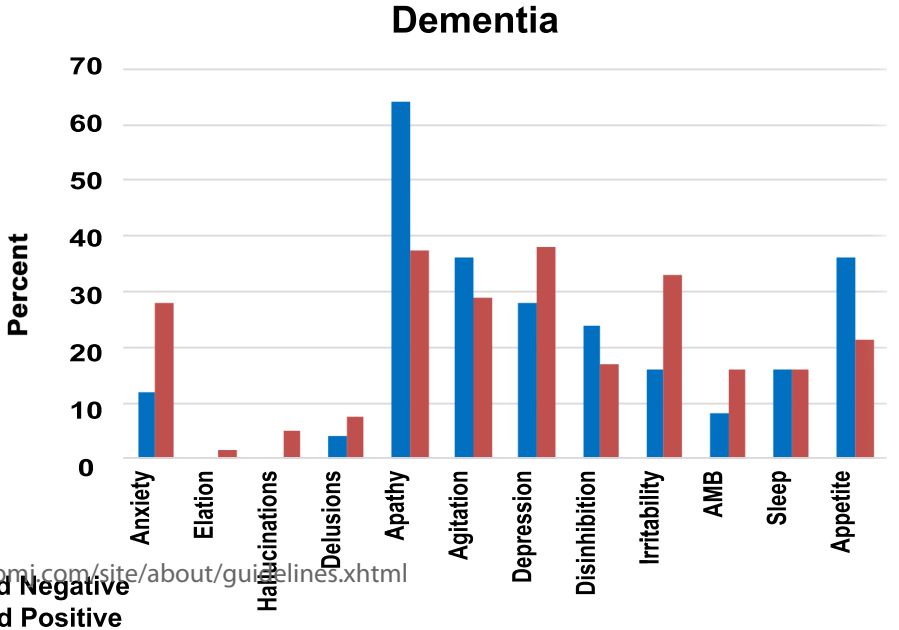
Figure 4: Survival curves showing the effect of the presence of the five most frequent early NPS on time to conversion from MCI to dementia

Figure 5: Survival curves showing the effect of de novo development of the five most frequent early NPS on time to conversion from MCI to dementia

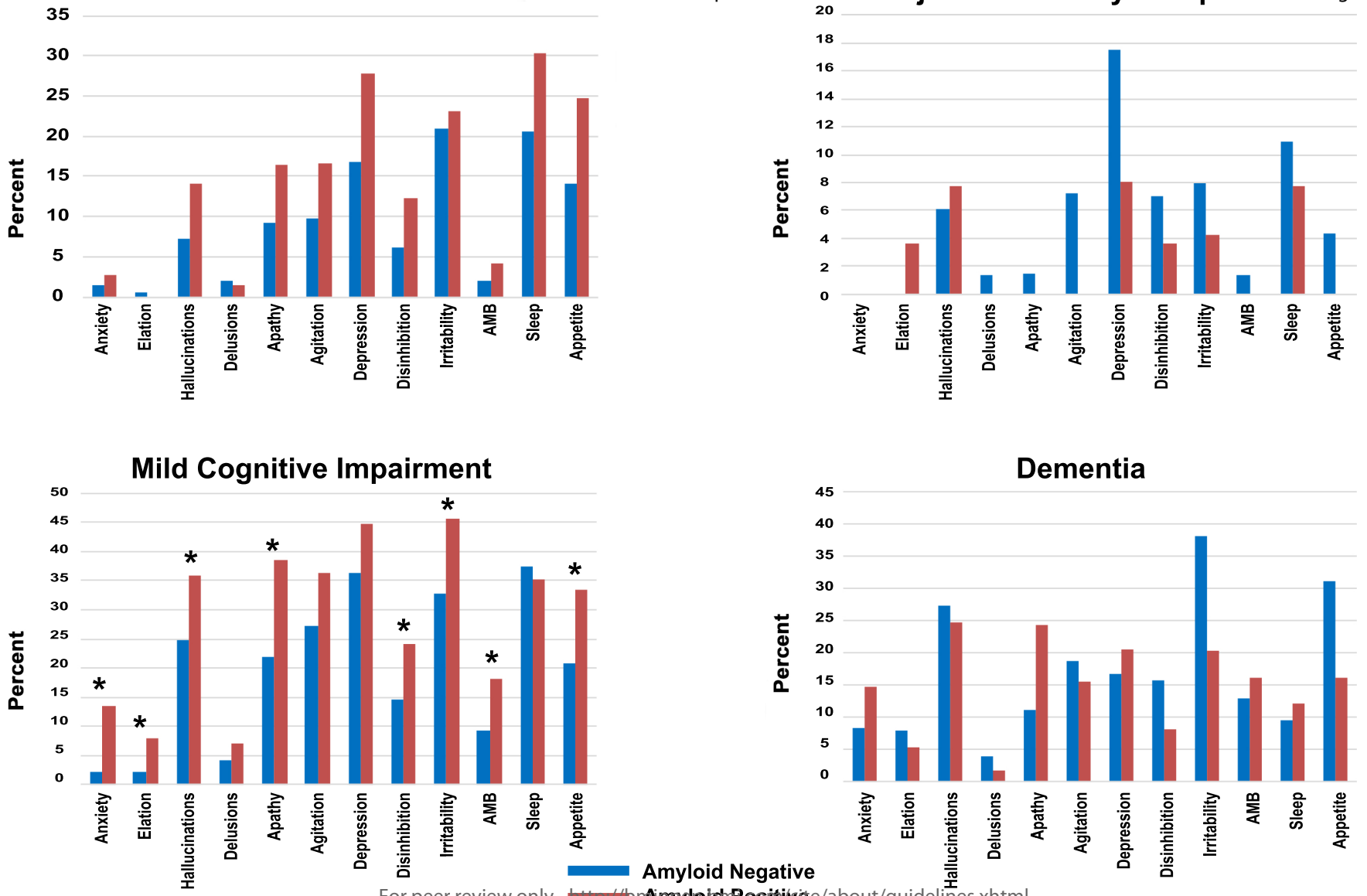


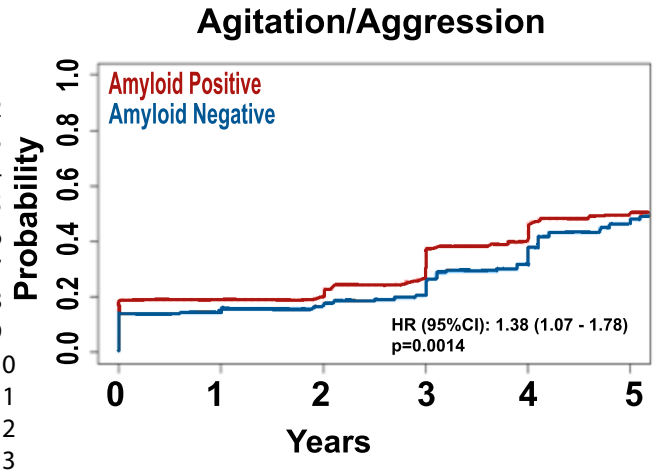


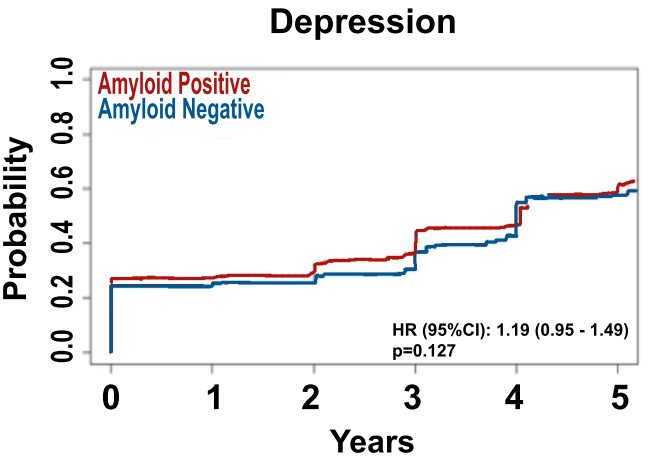


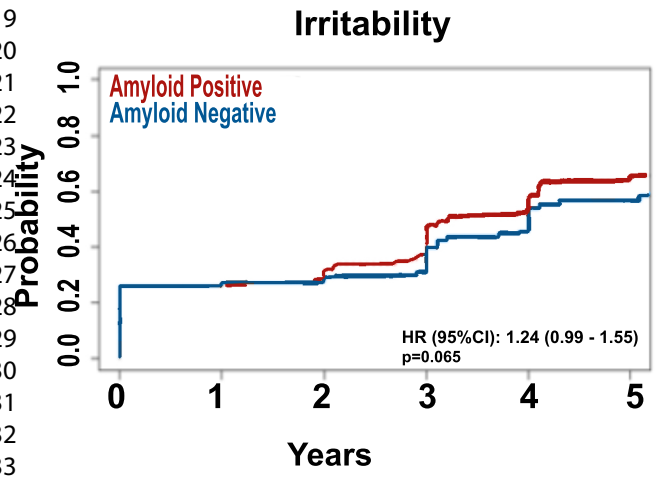


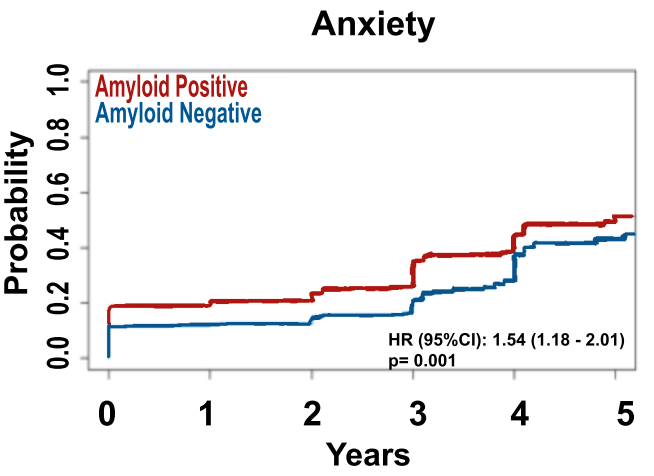
Amyloid Negative
Amyloid Positive
AMB = Aberrant Motor Behavior

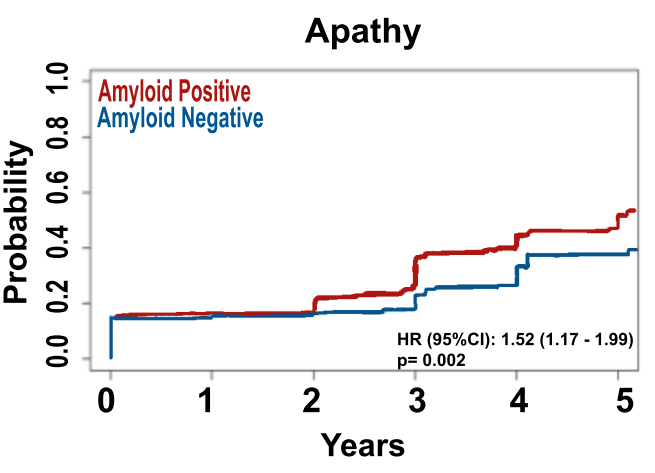


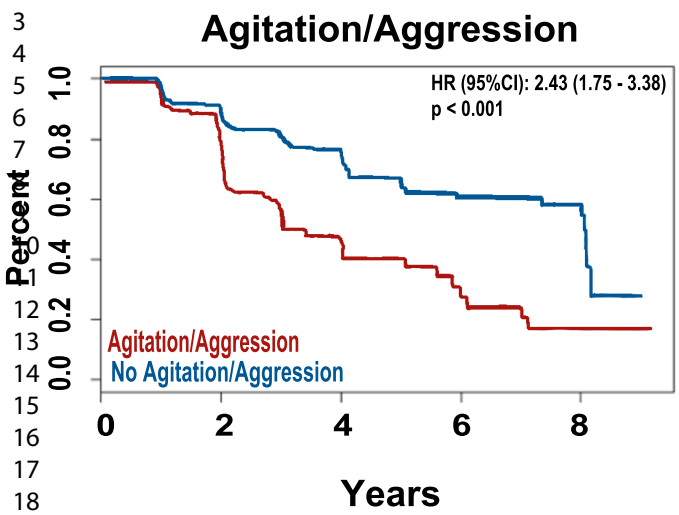

Agitation/Aggression
Amyloid Positive
Amyloid Negative
Probability
0.0 0.2 0.4 0.6 0.8 1.0
HR (95%CI): 1.38 (1.07 - 1.78)
p=0.0014
0 1 2 3 4 5
Years

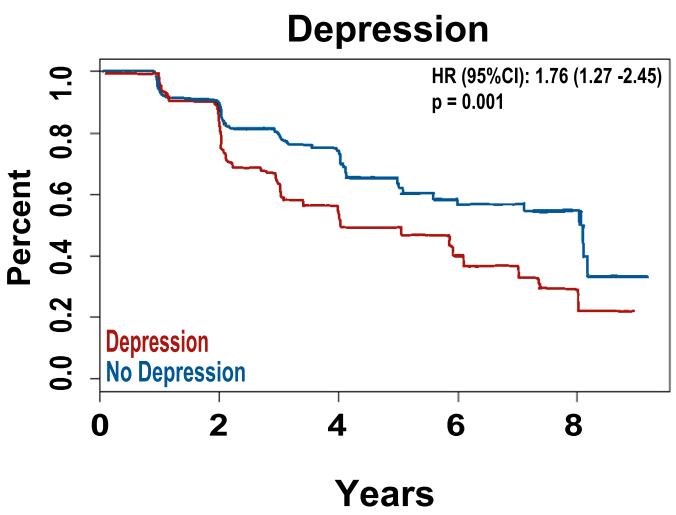

Depression
Amyloid Positive
Amyloid Negative
Probability
0.0 0.2 0.4 0.6 0.8 1.0
HR (95%CI): 1.19 (0.95 - 1.49)
p=0.127
0 1 2 3 4 5
Years

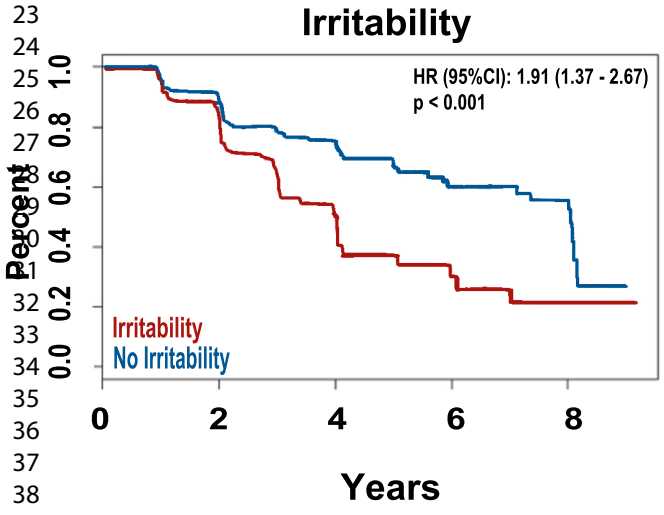

Irritability
Amyloid Positive
Amyloid Negative
Probability
0.0 0.2 0.4 0.6 0.8 1.0
HR (95%CI): 1.24 (0.99 - 1.55)
p=0.065
0 1 2 3 4 5
Years

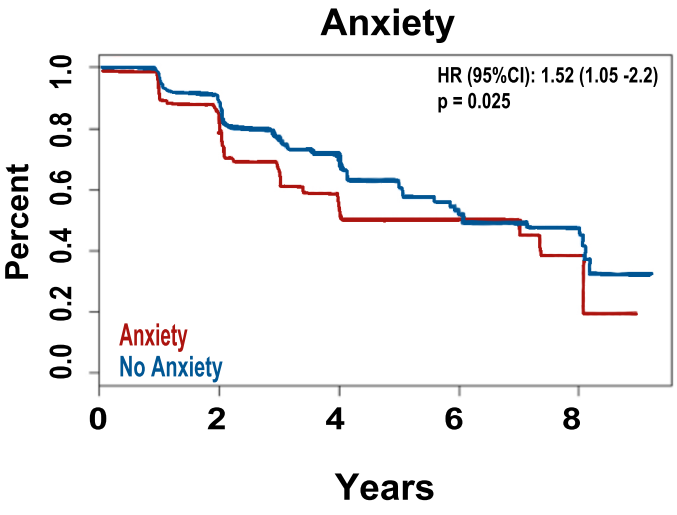

Anxiety
Amyloid Positive
Amyloid Negative
Probability
0.0 0.2 0.4 0.6 0.8 1.0
HR (95%CI): 1.54 (1.18 - 2.01)
p= 0.001
0 1 2 3 4 5
Years

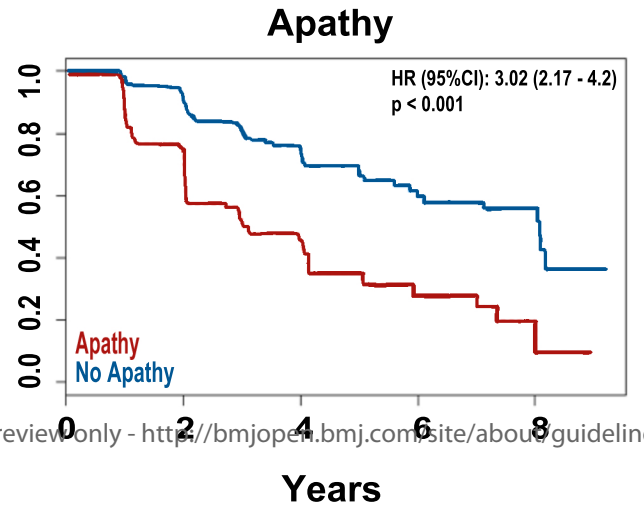

Apathy
Amyloid Positive
Amyloid Negative
Probability
0.0 0.2 0.4 0.6 0.8 1.0
HR (95%CI): 1.52 (1.17 - 1.99)
p= 0.002
0 1 2 3 4 5
Years

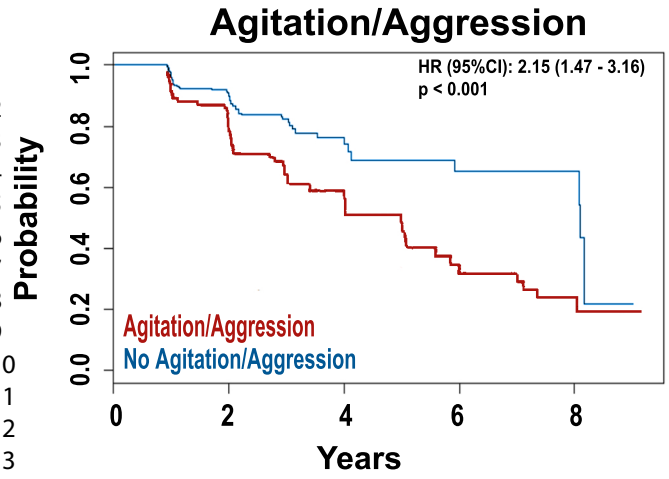


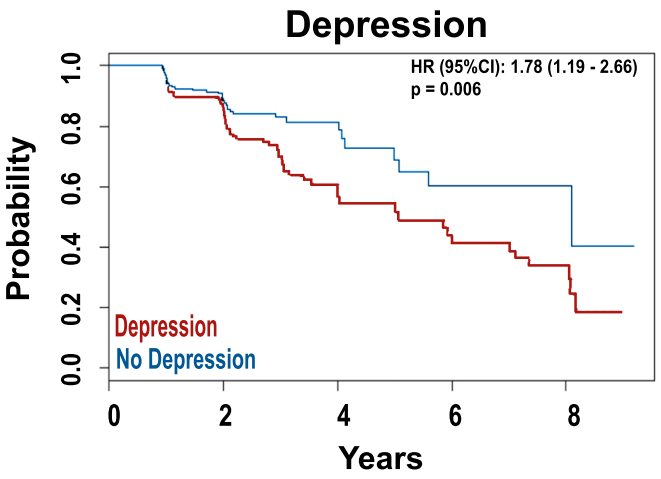


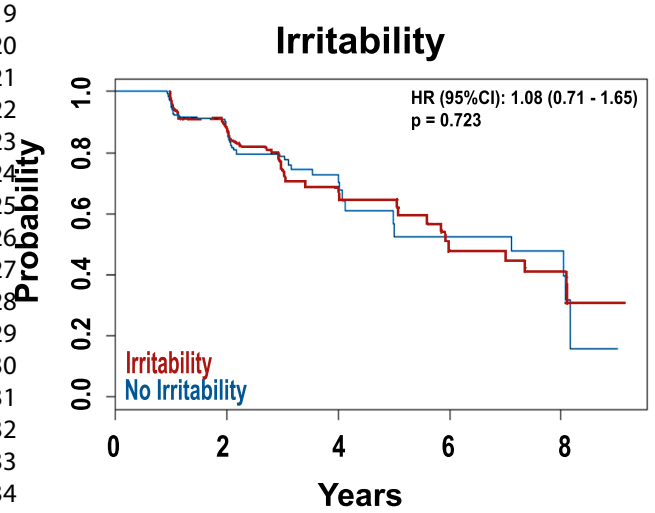


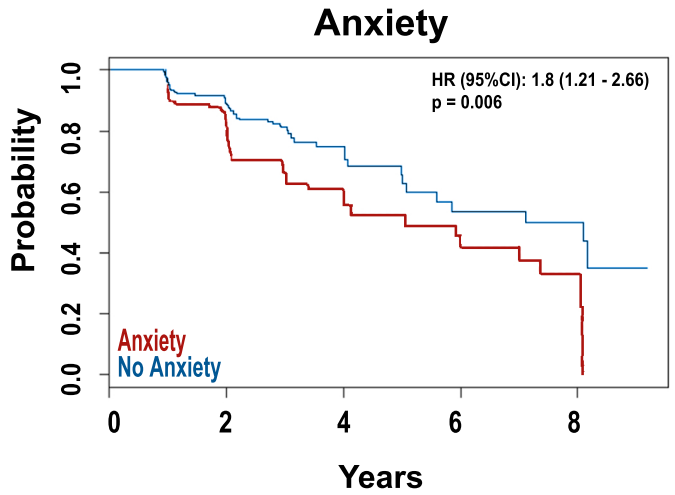


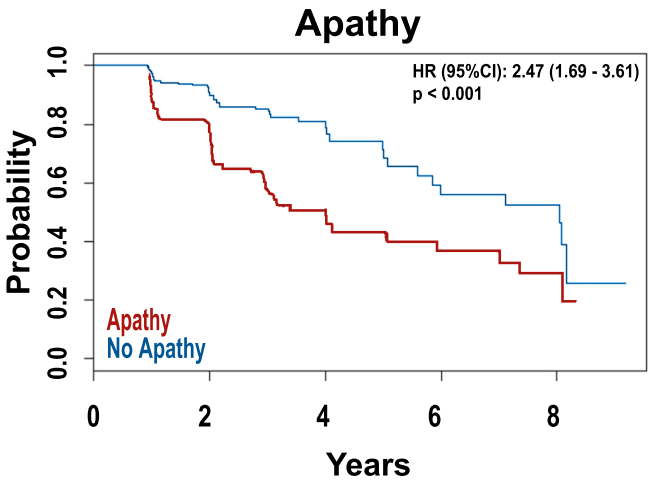



Agitation/Aggression
HR (95%CI): 2.15 (1.47 - 3.16)
p < 0.001
Agitation/Aggression
No Agitation/Aggression
Probability
0.0 0.2 0.4 0.6 0.8 1.0
0 2 4 6 8
Years


Depression
HR (95%CI): 1.78 (1.19 - 2.66)
p = 0.006
Depression
No Depression
Probability
0.0 0.2 0.4 0.6 0.8 1.0
0 2 4 6 8
Years


Irritability
HR (95%CI): 1.08 (0.71 - 1.65)
p = 0.723
Irritability
No Irritability
Probability
0.0 0.2 0.4 0.6 0.8 1.0
0 2 4 6 8
Years


Anxiety
HR (95%CI): 1.8 (1.21 - 2.66)
p = 0.006
Anxiety
No Anxiety
Probability
0.0 0.2 0.4 0.6 0.8 1.0
0 2 4 6 8
Years


Apathy
HR (95%CI): 2.47 (1.69 - 3.61)
p < 0.001
Apathy
No Apathy
Probability
0.0 0.2 0.4 0.6 0.8 1.0
0 2 4 6 8
Years

Supplemental Table 1

Variables		NC (N=275)				SMC (N=100)				MCI (N=559)				DEMENTIA (N=143)			
		Amyloid- (N=202)	Amyloid+ (N=73)	OR	p-value	Amyloid- (N=72)	Amyloid+ (N=28)	OR	p-value	Amyloid- (N=269)	Amyloid+ (N=290)	OR	p-value	Amyloid- (N=25)	Amyloid+ (N=118)	OR	p-value
Anxiety	**Yes**	10 (5)	2 (2.7)	0.54 (0.12, 2.52)	NS	6 (8.3)	2 (7.1)	0.85 (0.16, 4.49)	NS	31 (11.5)	55 (19)	1.8 (1.12, 2.9)	NS	3 (12)	33 (28)	2.85 (0.8, 10.16)	NS
Elation	**Yes**	0 (0)	0 (0)	∞	NS	0 (0)	0 (0)	∞	NS	9 (3.3)	7 (2.4)	0.71 (0.26, 1.93)	NS	0 (0)	2 (1.7)	∞	NS
Hallucinations	**Yes**	1 (0.5)	0 (0)	∞	NS	0 (0)	0 (0)	∞	NS	1 (0.4)	3 (1)	2.8 (0.29, 27.08)	NS	0 (0)	6 (5.1)	∞	NS
Delusions	**Yes**	0 (0)	0 (0)	∞	NS	0 (0)	0 (0)	∞	NS	0 (0)	7 (2.4)	∞	NS	1 (4)	9 (7.6)	1.98 (0.24, 16.38)	NS
Apathy	**Yes**	6 (3)	0 (0)	∞	NS	4 (5.6)	1 (3.6)	0.63 (0.07, 5.9)	NS	40 (14.9)	45 (15.5)	1.05 (0.66, 1.67)	NS	16 (64)	44 (37.3)	0.33 (0.13, 0.81)	NS
Agitation	**Yes**	9 (4.5)	1 (1.4)	0.3 (0.04, 2.41)	NS	3 (4.2)	3 (10.7)	2.76 (0.52, 14.58)	NS	38 (14.1)	55 (19)	1.42 (0.9, 2.23)	NS	9 (36)	34 (28.8)	0.72 (0.29, 1.79)	NS
Depression	**Yes**	17 (8.4)	1 (1.4)	0.15 (0.02, 1.15)	NS	9 (12.5)	3 (10.7)	0.84 (0.21, 3.36)	NS	65 (24.2)	78 (26.9)	1.15 (0.79, 1.68)	NS	7 (28)	45 (38.1)	1.59 (0.62, 4.11)	NS
Disinhibition	**Yes**	5 (2.5)	0 (0)	∞	NS	1 (1.4)	0 (0)	∞	NS	24 (8.9)	30 (10.3)	1.18 (0.67, 2.07)	NS	6 (24)	20 (16.9)	0.65 (0.23, 1.83)	NS
Irritability	**Yes**	20 (9.9)	4 (5.5)	0.53 (0.17, 1.61)	NS	9 (12.5)	4 (14.3)	1.17 (0.33, 4.16)	NS	70 (26)	76 (26.2)	1.01 (0.69, 1.47)	NS	4 (16)	39 (33.1)	2.59 (0.83, 8.07)	NS
Aberrant motor behavior	**Yes**	2 (1)	0 (0)	∞	NS	1 (1.4)	0 (0)	∞	NS	7 (2.6)	13 (4.5)	1.76 (0.69, 4.48)	NS	2 (8)	19 (16.1)	2.21 (0.48, 10.17)	NS
Sleep disturbance	**Yes**	22 (10.9)	7 (9.6)	0.87 (0.36, 2.13)	NS	17 (23.6)	2 (7.1)	0.25 (0.05, 1.16)	NS	55 (20.4)	59 (20.3)	0.99 (0.66, 1.49)	NS	4 (16)	19 (16.1)	1.01 (0.31, 3.28)	NS
Appetite changes	**Yes**	3 (1.5)	0 (0)	∞	NS	3 (4.2)	1 (3.6)	0.85 (0.08, 8.53)	NS	25 (9.3)	21 (7.2)	0.76 (0.41, 1.39)	NS	9 (36)	25 (21.2)	0.48 (0.19, 1.21)	NS

Supplemental Table 2												
Variables	**NC (N=275)**			**SMC (N=100)**			**MCI (N=559)**			**DEMENTIA (N=143)**		
	Amyloid- (N=202)	Amyloid+ (N=73)	p-value	Amyloid- (N=72)	Amyloid+ (N=28)	p-value	Amyloid- (N=269)	Amyloid+ (N=290)	p-value	Amyloid- (N=25)	Amyloid+ (N=118)	p-value
Anxiety	0.11 (0.59)	0.10 (0.59)	NS	0.21 (1.01)	0.18 (0.77)	NS	0.31 (1.14)	0.57 (1.44)	**0.023**	0.38 (1.14)	0.70 (1.39)	NS
Elation	0 (0)	0 (0)	∞	0 (0)	0 (0)	∞	0.08 (0.51)	0.09 (0.67)	NS	0 (0)	0.08 (0.74)	NS
Hallucinations	0 (0)	0 (0)	∞	0 (0)	0 (0)	∞	0.02 (0.26)	0.07 (0.59)	NS	0 (0)	0.07 (0.31)	NS
Delusions	0 (0)	0 (0)	∞	0 (0)	0 (0)	∞	0.004 (0.06)	0.14 (0.64)	**0.001**	0.13 (0.61)	0.36 (1.45)	NS
Apathy	0.11 (0.89)	0.24 (1.19)	NS	0.14 (0.59)	0.11 (0.57)	NS	0.50 (1.61)	0.63 (1.73)	NS	2.58 (2.87)	1.27 (2.14)	**0.011**
Agitation	0.14 (0.95)	0.10 (0.42)	NS	0.06 (0.29)	0.18 (0.61)	NS	0.39 (1.11)	0.56 (1.35)	NS	1.71 (2.85)	0.62 (1.27)	**0.003**
Depression	0.23 (0.92)	0.18 (0.83)	NS	0.24 (0.83)	0.11 (0.32)	NS	0.64 (1.41)	0.72 (1.38)	NS	0.67 (1.24)	0.80 (1.27)	NS
Disinhibition	0.04 (0.21)	0.13 (0.75)	NS	0.03 (0.24)	0 (0)	NS	0.25 (1.02)	0.31 (1.11)	NS	0.71 (1.37)	0.32 (1.04)	NS
Irritability	0.21 (0.88)	0.29 (0.86)	NS	0.26 (0.79)	0.25 (0.65)	NS	0.78 (1.78)	0.75 (1.54)	NS	0.63 (1.61)	1.02 (2.063)	NS
Aberrant motor behavior	0.01 (0.07)	0.08 (0.52)	NS	0.04 (0.35)	0 (0)	NS	0.09 (0.64)	0.29 (1.28)	**0.025**	0.29 (1.0)	0.59 (1.62)	NS
Sleep disturbance	0.38 (1.29)	0.49 (1.28)	NS	0.57 (1.23)	0.25 (0.93)	NS	0.85 (1.94)	0.99 (2.26)	NS	0.46 (1.14)	0.59 (1.58)	NS
Appetite changes	0.10 (0.71)	0.24 (1.22)	NS	0.21 (1.17)	0.04 (0.19)	NS	0.29 (1.22)	0.51 (1.70)	0.084	2.42 (3.86)	0.86 (2.1)	**0.006**

Supplemental Table 3

Variables		NC (N=275)			SMC (N=100)			MCI (N=559)			DEMENTIA (N=143)		
		Amyloid- (N=202)	Amyloid+ (N=73)	p-value	Amyloid- (N=72)	Amyloid+ (N=28)	p-value	Amyloid- (N=269)	Amyloid+ (N=290)	p-value	Amyloid- (N=25)	Amyloid+ (N=118)	p-value
Anxiety	**Yes**	3 (1.5)	2 (2.7)	NS	0 (0)	0 (0)	NS	6 (2.2)	38 (13.4)	**< 0.001**	2 (8.3)	16 (14.7)	NS
Elation	**Yes**	1 (0.5)	0 (0)	NS	0 (0)	1 (3.6)	NS	6 (2.2)	23 (8)	**0.007**	2 (8)	6 (5.4)	NS
Hallucinations	**Yes**	14 (7.3)	10 (14.1)	NS	4 (6.1)	2 (7.7)	NS	59 (24.8)	84 (35.9)	**0.014**	6 (27.3)	21 (24.7)	NS
Delusions	**Yes**	4 (2)	1 (1.4)	NS	1 (1.4)	0 (0)	NS	11 (4.2)	20 (7.1)	NS	1 (4)	2 (1.7)	NS
Apathy	**Yes**	18 (9.2)	12 (16.4)	NS	1 (1.5)	0 (0)	NS	50 (21.8)	94 (38.4)	**< 0.001**	1 (11.1)	18 (24.3)	NS
Agitation	**Yes**	19 (9.8)	12 (16.7)	NS	5 (7.2)	0 (0)	NS	63 (27.3)	85 (36.2)	0.063	3 (18.8)	13 (15.5)	NS
Depression	**Yes**	31 (16.8)	20 (27.8)	NS	11 (17.5)	2 (8)	NS	74 (36.3)	95 (44.8)	NS	3 (16.7)	15 (20.5)	NS
Disinhibition	**Yes**	12 (6.1)	9 (12.3)	NS	5 (7)	1 (3.6)	NS	36 (14.7)	63 (24.2)	**0.014**	3 (15.8)	8 (8.2)	NS
Irritability	**Yes**	38 (20.9)	16 (23.2)	NS	5 (7.9)	1 (4.2)	NS	65 (32.7)	97 (45.5)	**0.014**	8 (38.1)	16 (20.3)	NS
Aberrant motor behavior	**Yes**	4 (2)	3 (4.1)	NS	1 (1.4)	0 (0)	NS	24 (9.2)	50 (18.1)	**0.00**8	3 (13)	16 (16.2)	NS
Sleep disturbance	**Yes**	37 (20.6)	20 (30.3)	NS	6 (10.9)	2 (7.7)	NS	80 (37.4)	81 (35.2)	NS	2 (9.5)	12 (12.1)	NS
Appetite changes	**Yes**	28 (14.1)	18 (24.7)	NS	3 (4.3)	0 (0)	NS	51 (20.9)	90 (33.5)	**0.007**	5 (31.2)	15 (16.1)	NS

BMJ Open

The Association of Brain Amyloidosis with the incidence and frequency of Neuropsychiatric Symptoms in ADNI - a multisite observational cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031947.R1
Article Type:	Original research
Date Submitted by the Author:	08-Oct-2019
Complete List of Authors:	Goukasian, Naira; University of Vermont College of Medicine Hwang, Kristy; Emory University School of Medicine Romero, Tamineh; University of California Los Angeles, Medicine Statistics Core Grotts, Jonathan; University of California Los Angeles, Medicine Statistics Core Do, Triet; Tulane University School of Medicine Groh, Jenna; Indiana University School of Medicine, Neurology Bateman, Daniel; Indiana University School of Medicine, Psychiatry Apostolova, Liana; Indiana University School of Medicine, Neurology
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Neurology
Keywords:	Amyloidosis; Mild Cognitive Impairment (MCI), Alzheimer's disease (AD), Neuropsychiatric Symptoms (NPS), Alzheimer's Disease Neuroimaging Initiative (ADNI)

The Association of Brain Amyloidosis with the incidence and frequency of Neuropsychiatric Symptoms in ADNI - a multisite observational cohort study

Naira Goukasian[1], Kristy S. Hwang[2], Tamineh Romero[3], Jonathan Grotts[3], Triet M. Do[4], Jenna Groh [6], Daniel R. Bateman[5], Liana G. Apostolova [6,7,8] for the Alzheimer's Disease Neuroimaging Initiative*

* Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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 or in the provided supplementary ADNI Coinvestigator Appendix

[1] University of Vermont Larner College of Medicine, Burlington, VT, USA
[2] Emory University School of Medicine, Atlanta, GA, USA
[3] Department of Medicine Statistics Core, University of California, Los Angeles, CA, USA
[4] Tulane University Medical School, New Orleans, LA, USA
[5] Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA
[6] Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, USA
[7] Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, USA
[8] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

Submitted: **10/2/2019**

Send correspondence to:
Liana Apostolova, MD, MSc, FAAN
Department of Neurology
Indiana University School of Medicine
355 W. 16th Street, Suite 4019
Indianapolis, IN 46202
Phone: +1 317/963-7436
Fax: +1 317/963-7533
E-mail: lapostol@iu.edu

Word count: Abstract 239, Body 4,151
Title: 122 characters (including spaces)
References: 47
Figures: 4
Tables: 1
Supplemental Tables: 3

KEYWORDS

Amyloidosis; Mild Cognitive Impairment (MCI); Alzheimer's disease (AD); Neuropsychiatric Symptoms (NPS); Alzheimer's Disease Neuroimaging Initiative (ADNI)

AUTHOR CONTRIBUTIONS

- Naira Goukasian assisted with data processing and analyses, and the completion of integral statistical analyses. Ms. Goukasian was the primary author responsible for drafting the manuscript.

- Kristy S. Hwang assisted with data processing and analyses, provided critical insights for interpretation of the results and participated in revising of the manuscript.

- Tamineh Romero completed integral statistical analyses, provided critical insights for interpretation of our results and participated in revising of the manuscript.

- Jonathan Grotts completed integral statistical analyses, provided critical insights for interpretation of our results and participated in revising of the manuscript.

- Triet M. Do completed some of the analyses and took part in revising of the manuscript.

- Jenna Groh assisted in editing of figures and participated in revising of the manuscript

- Daniel R. Bateman provided critical insights for interpretation of our results and participated in revising of the manuscript.

- Liana G. Apostolova was responsible for the study concept and design. She provided significant oversight over all analyses, interpretation of results, and participated in all stages of manuscript preparation.

FINANCIAL DISCLOSURES

- Naira Goukasian has no disclosures.

- Kristy S. Hwang has no disclosures.

- Jonathan Grotts has no disclosures

- Tamineh Romero has no disclosures

- Triet M. Do has no disclosures.

- Jenna Groh has no disclosures

- Daniel R. Bateman has no disclosures.

- Liana G. Apostolova received research support from General Electric Healthcare, Piramal and Eli Lilly, served on the speaker's bureau for Eli Lilly & Company and Piramal Enterprises and on an advisory board for Eli Lilly & Company.

FUNDING:

This work was generously supported by NIA R01 AG040770, NIA K02 AG048240, NIA P50 AG16570, NIA P30 AG010133, NIA U01 AG024904 and the Easton Consortium for Alzheimer's Drug Discovery and Biomarker Development.

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical

sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

DATA AVAILABILITY:

Data used in this manuscript 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 only some participated in analysis or writing of this report (complete listing available at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf).

ACKNOWLEDGEMNTS:

We would like to acknowledge and thank Dr. David Elashoff from the Department of Medicine Statistics Core at the University of California, Los Angeles for his assistance with the statistical design of this manuscript.

ABSTRACT

Objective: To investigate the relationship between amyloid burden and frequency of existing and incidence of new neuropsychiatric symptoms (NPS) in elderly with and without cognitive decline.

Methods: 275 cognitively normal (NC), 100 subjective memory complaint (SMC), 559 MCI, and 143 AD dementia Alzheimer's Disease Neuroimaging Initiative subjects received [^{18}F]-Florbetapir PET scans. Yearly neuropsychiatric inventory (NPI/NPI-Q) data were collected from the study partners at each visit. Mean standard uptake volume ratios (SUVR) normalized to whole cerebellum were obtained. Positive amyloid PET scan was defined as mean SUVR ≥1.17. Fisher-exact test was used to compare NPS frequency and incidence between amyloid positive and amyloid negative subjects. Survival analyses were used to estimate hazard ratios for developing the most common NPS by amyloid status.

Results: No differences in NPS frequency were seen between amyloid positive and amyloid negative NC, SMC, MCI, or dementia groups. MCI subjects with amyloid pathology however tended to have greater frequency*severity (FxS) of all NPS except for agitation, depression, nighttime disturbances and elation. MCI subjects with amyloid pathology were at greater risk for developing apathy, anxiety and agitation over time. Baseline presence of agitation and apathy and new onset agitation, irritability and apathy predicted faster conversion to dementia among MCI subjects.

Conclusions: Amyloid pathology is associated with greater rate of development of new NPS in MCI. Anxiety and delusions are significant predictors of amyloid pathology. Agitation, irritability and apathy are significant predictors for conversion from MCI to dementia.

ARTICLE SUMMARY

Strength and limitations of this study

- Our analyses used ADNI data; ADNI - the premier longitudinal biomarker study in AD, employs standardized subject assessment and MRI and PET scan collection protocols and meticulous data quality control across all study sites.
- A strength of our study is the relatively large sample size and the inclusion of all diagnostic groups across the AD spectrum including cognitively normal participants many of which are in the presymptomatic AD stages, as well as the use of neuropsychiatric data collection tools that are administered to the caregivers and not the subject themselves.
- One of the limitations of this study is that the NPI and NPI-Q use structured questions focused on the frequency and severity of symptoms from the preceding month only. Therefore, intermittent NPS that were not manifested by the subjects in the pre-specified timeframe are not adequately captured.
- Another limitation to our study is that ADNI employs rigorous exclusion criteria typical of clinical trials and hence our study cohort might not be representative of the general population.
- ADNI excludes subjects with preexisting depression (Geriatric depression scale score >5) which likewise could affect the generalization of the results.

BACKGROUND

Alzheimer's disease (AD) is the most common form of dementia, accounting for 60-80% of all dementia cases. AD is characterized by cortical amyloid plaque and neurofibrillary tangle deposition, as well as progressive synaptic and neuronal loss. Recently developed amyloid positron emission tomography (PET) radioligands with high affinity for amyloid plaques, such as ^{18}F-Florbetaben, ^{18}F-Flutemetamol, and ^{18}F-Florbetapir, can provide reliable *in vivo* visualization of cortical fibrillary β-amyloid plaque deposition [1-3]. With amyloid accumulation beginning up to two decades prior to symptoms onset, these amyloid PET radiotracers can measure amyloid burden in the symptomatic, as well as the asymptomatic stages [4 5].

Neuropsychiatric symptoms (NPS) are prominent features of AD and mild cognitive impairment (MCI). 35-75% of MCI subjects experience at least one neuropsychiatric symptom with depression, apathy, and anxiety being the most prevalent [6-8]. In the dementia stage, apathy, agitation, and anxiety are most prevalent, followed by aberrant motor behavior, dysphoria and disinhibition [7 9].

NPS have been associated with higher likelihood for cognitive decline. Symptoms of depression, irritability, and agitation were found to predict cognitive decline among cognitively normal, MCI, as well as individuals with subjective cognitive concerns pooled together[10]. One population-based study found that the presence of symptoms such as agitation, apathy, anxiety, irritability, and depression in normal controls (NC) at baseline significantly predicted incident MCI [11]. Neuroticism and its underlying facets of anxiety, depression and stress were

associated with diagnosis of MCI and neurodegeneration [12]. Other studies have shown that the presence of one or more NPS in MCI, specifically symptoms of agitation, depression, and apathy, increase the risk of progression from MCI to AD dementia [10 13-19]. Most patients with AD experience at least one NPS during the course of disease progression [6]. NPS in dementia are associated with faster disease progression [20 21], increased mortality [15], and caregiver burden [22], as well as shorter time until nursing home admission [23 24].

To date a few groups have studied the associations between NPS and amyloid burden and some have suggested that NPS reflect underlying amyloid pathology[25 26]. Studies have gone on to show that increased apathy was significantly associated with greater cortical amyloid burden in MCI [27]. Anxious MCI subjects were 3.1 times more likely to have abnormal cerebrospinal fluid amyloid β levels – a proxy marker for brain amyloidosis [28]. Another group found both anxiety and irritability to associate with greater amyloid burden across the AD spectrum [29]. Amyloid positive NC who reported anxiety experienced faster cognitive decline [30] and greater frequency of mood disturbances over the following year [31]. Taken together these data seem to suggest that at least some NPS are associated with amyloid pathology and cognitive decline.

Here we investigate the relationship between amyloid burden and incidence and frequency of NPS across the spectrum from normal cognition to dementia. We hypothesized that amyloid pathology will associate with higher incidence of NPS across all disease stages. We postulated that amyloid deposition will associate with apathy, anxiety, irritability and depression in the asymptomatic and early

symptomatic stages, and with psychosis, aberrant motor behaviors, disinhibition and agitation in the late symptomatic stages.

METHODS

Subjects:

Data used in these analyses were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD).

The first phase of ADNI, ADNI-1, recruited approximately 800 adults, ages 55 to 90 across 50 sites in the United States and Canada. The study sample consisted of approximately 200 older NC individuals, 400 people with late amnestic MCI (LMCI) and 200 people with mild AD. ADNI expanded enrollment criteria with the launch of ADNI-GO in 2009 and enrolled 200 additional subjects with early amnestic MCI (EMCI). ADNI-2 added approximately 650 newly enrolled subjects [150 NC, 100 subjects with subjective memory complaints (SMC), 100 EMCI, 150 LMCI and 150 mild AD]. All procedures were approved by the Institutional Review Boards of all participating institutions. Written informed consent was obtained from every research participant according to the Declaration of Helsinki and the Belmont Report.

The clinical description of the ADNI cohort has been previously published [32]. Diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association (NINCDS-ADRDA) criteria [33]. AD subjects were required to have Mini Mental State Examination (MMSE) [34] scores between 20 and 26 and a Clinical Dementia Rating scale (CDR) [35] score of 0.5–1 at baseline. Qualifying MCI subjects had memory complaints, but no significant functional impairment, scored between 24 and 30 on the MMSE, had a global CDR score of 0.5, a CDR memory score of 0.5 or greater, and objective memory impairment on Wechsler Memory Scale – Logical Memory II test (WMS-LMII) [36]. NC and SMC subjects had MMSE scores between 24 and 30, a global CDR of 0 and did not meet criteria for MCI and AD. NC were devoid of cognitive concerns, while SMC subjects had significant memory concerns manifested in a score of ≥ 16 on the first 12 items of the Cognitive Change Index [37]. Subjects were excluded if they refused or were unable to undergo MRI, had other neurological disorders, active depression, or history of psychiatric diagnosis, alcohol, or substance dependence within the past 2 years, less than 6 years of education, or were not fluent in English or Spanish. Inclusion and diagnostic criteria, as well as procedures and protocols, for the ADNI studies can be found on http://www.adni-info.org/Scientists/ADNIStudyProcedures.html. Written informed consent was obtained from all participants. For more up-to-date information, see www.adni-info.org.

[^{18}F]-Florbetapir PET amyloid imaging was added in the ADNI-GO/2 stages of the study. We downloaded the clinical, behavioral and amyloid PET data of 275

NC, 100 SMC, 559 MCI and 143 AD subjects from the ADNI-1, ADNI-GO, and ADNI-2 databases on November 3, 2015. EMCI and LMCI were grouped in our analyses.

Neuropsychiatric Data:

Neuropsychiatric data were captured with the Neuropsychiatric Inventory (NPI) [38] and the NPI Questionnaire (NPI-Q) [39]. ADNI-1 used the NPI-Q while ADNI-GO/2 used the full version. Both versions assess twelve symptoms - delusions, hallucinations, agitation, anxiety, apathy, irritability, depression, euphoria, disinhibition, aberrant motor behavior, sleep and appetite. Interviewers ask structured questions about the presence and severity (as well as frequency in the full version of the NPI, but not in NPI-Q) of the symptoms in the past month to the study partner. Those enrolled in ADNI-1 only had the NPI-Q available, while those enrolled in ADNI-2 and ADNI-GO were administered the full version.

NPI or NPI-Q data from the baseline and all annual visits were obtained from LONI IDA on November 3, 2015. Each patient had either NPI or NPI-Q for each visit that was analyzed. NPS at baseline were coded as “absent” if not endorsed and as “present” if endorsed by the study partner. In our longitudinal analyses, we coded symptoms in follow-up as “absent” if never endorsed by the study partner (including the baseline visit) and as “emerging de novo” if absent at baseline but reported at one or more follow-up visit. Frequency x severity (FxS) scores were obtained for subjects that received the full NPI questionnaire at baseline.

Imaging Data and Analysis:

A detailed description of AV-45 PET acquisition may be found at http://www.adni-info.org/Scientists/ADNIStudyProcedures.html. Briefly, 370 MBq (10 mCi +/- 10%) bolus injection of AV-45 was administered intravenously. Approximately 50 minutes after injection, a 20-minute continuous brain PET imaging session collected a dynamic amyloid PET data consisting of four 5-minute frames. In our main analyses, we used the mean whole brain Standard Uptake Volume Ratios (SUVR) from University of California Berkeley (UCB) downloaded from ADNI's database on November 3, 2015. The mean whole brain SUVR was obtained by averaging the SUVR values across the frontal, anterior/posterior cingulate, lateral parietal and lateral temporal grey matter regions [40]. The UCB protocols for ^{18}F-Florbetapir preprocessing, co-registration and normalization have been previously described [40]. We defined a positive amyloid PET scan as mean SUVR ≥1.17 [41].

Statistical Analyses:

Demographic comparisons between amyloid positive and amyloid negative groups within diagnostic categories were done using Fisher-exact for categorical variables and Wilcoxon rank-sum test for continuous variables. Comparisons of the frequency of symptoms at baseline, as well as the emergence of new NPS in follow-up conditional on amyloid status were done using Fisher-exact test. Using stepwise backwards logistical regression we also studied the predictive value of

the presence/absence of NPI behaviors as well as their FxS scores on amyloid status while adjusting for age, sex, education and *APOE4*.

Survival analyses using Cox proportional hazard regression models were used to determine 1) the hazard ratios for developing the five most common early NPI symptoms – apathy, anxiety, agitation, irritability, and depression – in amyloid positive versus amyloid negative participants in the NC, subjective memory complaints (SMC) and MCI groups; 2) the hazard for conversion from MCI to dementia in all MCI subjects based on the presence of the top five most common early NPI symptoms at baseline; and 3) the hazard for conversion from MCI to dementia in MCI subjects who were free of the five most common NPI symptoms at baseline but developed them in follow-up. For analyses 2) and 3), subsequent visits were excluded once a subject was diagnosed with dementia. Subjects who reverted from MCI to NC (N=34) in follow-up were excluded from all analyses. We censored the data ignoring visits after dementia was diagnosed in our time to conversion Cox proportional hazard regression models. All Cox proportional hazard regression models were adjusted for age, education, and *APOE4* status. The Cox regression models were repeated while additionally adjusting for amyloid status. P-values were adjusted for multiple comparisons using FDR. All Cox regression models were evaluated for proportional hazard assumption and there was no evidence that the models did not meet required assumptions (q-value>0.081). All p-values were adjusted for multiple comparison correction using Benjamini & Hochberg[42] false discovery rate (FDR) correction. All statistical tests were two-sided and a q-value less than 0.05 was considered statistically

significant. All statistical analyses were done in the R Statistical Computing Environment (R Core Team, Vienna, Austria).

Patient and Public Involvement:

Our study represents secondary analyses of preexisting data. Patients or the public were not engaged in defining our research questions and outcome measures and have not provided input into study design and conduct of this study. A yearly manuscript summarizing all findings from the ADNI study is published by the ADNI leadership team [43-45]. As our analyses use deidentified data only study participants will not be contacted with these findings.

RESULTS

Our sample consisted of a total of 1,077 ADNI subjects including 275 NC (26.5% amyloid positive), 100 SMC (28.0% amyloid positive), 559 MCI (52.0% amyloid positive), and 143 AD (82.5% amyloid positive) subjects with average follow-up time of 3.2 ± 2.4 years (Table). Amyloid positive NC and SMC were significantly older and more likely to be Apolipoprotein E *ε4 (APOE4)* positive relative to their amyloid negative counterparts. Amyloid positive NC were also less educated and performed worse on the Alzheimer's Disease Assessment Scale – Cognition Subscale 11 (ADAScog-11) compared to amyloid negative NC. Amyloid positive MCI were significantly older, more likely to be *APOE4* positive, and showed greater impairment on Mini-Mental State Examination (MMSE), Clinical Dementia Rating – Sum of Boxes (CDR-SB), ADAScog-11, and Functional

Assessment Questionnaire (FAQ) scales compared to amyloid negative MCI. Amyloid positive dementia subjects were more likely to be *APOE4* positive, and showed greater impairment on the ADAScog-11 compared to the amyloid negative dementia subgroup. No significant difference in years of follow-up was seen between the amyloid positive vs. negative subjects in each of the diagnostic groups (**Table**).

Table: Demographic comparisons by amyloid status across the diagnostic groups

Variables	NC (N=275)			SMC (N=100)			MCI (N=559)			DEMENTIA (N=143)		
	Amyloid- (N=202)	Amyloid+ (N=73)	q-value*	Amyloid- (N=72)	Amyloid+ (N=28)	q-value*	Amyloid- (N=269)	Amyloid+ (N=290)	q-value*	Amyloid- (N=25)	Amyloid+ (N=118)	q-value*
Age, mean (SD)	73.3 (6.0)	76.1 (5.0)	**0.001**	71.5 (5.3)	74.6 (6.0)	**0.015**	70.7 (7.8)	73.1 (6.9)	**<0.001**	75.8 (7.2)	74.0 (8.3)	NS
Education, mean (SD)	16.7 (2.6)	15.9 (2.7)	**0.03**	16.9 (2.2)	16.4 (3.1)	NS	16.3 (2.6)	15.9 (2.8)	NS	16.4 (2.4)	15.7 (2.7)	NS
Gender, M/F %	52/48	41/59	NS	47/53	25/75	NS	56/44	58/42	NS	80/20	53/47	**0.015**
APOE ε4, 0/1/2 %	80/20/0	55/45/0	**<0.001**	81/19/0	46/54/0	**0.004**	76/24/0	29/68/3	**<0.001**	76/16/8	25/52/23	**<0.001**
MMSE, mean (SD)	29.0 (1.2)	29.2 (0.9)	NS	29.0 (1.3)	29.1 (0.8)	NS	28.4 (1.5)	27.6 (1.8)	**<0.001**	23.3 (2.4)	23.1 (2.1)	NS
CDR-SB, mean (SD)	0.03 (0.11)	0.04 (0.16)	NS	0.06 (0.16)	0.13 (0.22)	NS	1.3 (0.7)	1.6 (0.9)	**<0.001**	4.3 (1.8)	4.5 (1.7)	NS
ADAScog-11, mean (SD)	5.6 (3.0)	6.4 (3.0)	**0.048**	5.5 (2.8)	5.9 (2.6)	NS	8.0 (3.7)	10.5 (4.5)	**<0.001**	17.6 (6.2)	21.3 (7.4)	**0.019**
FAQ, mean (SD)	0.2 (0.7)	0.1 (0.5)	NS	0.6 (2.0)	0.5 (1.0)	NS	1.9 (3.2)	3.5 (4.2)	**<0.001**	12.3 (7.8)	13.2 (7.0)	NS
NPI total, mean (SD)	1.0 (2.5)	0.7 (1.2)	NS	1.8 (3.8)	1.1 (1.8)	NS	3.5 (6.0)	4.6 (6.7)	NS	10.0 (9.1)	7.3 (8.8)	NS
Years of follow-up, mean (SD)	4.0 (2.9)	4.3 (2.9)	NS	1.6 (0.8)	1.2 (1.0)	NS	3.6 (2.1)	3.5 (2.2)	NS	1.1 (0.9)	1.1 (0.7)	NS

NOTE: * q-values are adjusted for multiple comparison using FDR within each subset

Neuropsychiatric Symptoms at baseline:

No differences in NPS frequency at baseline were seen between amyloid positive and amyloid negative NC, SMC, MCI, or dementia groups **(Supplementary Table 1)**. Comparing NPI FxS means between the amyloid positive and negative subgroups within each diagnostic group revealed significantly greater aberrant motor behaviors FxS in amyloid positive vs. amyloid negative NC (0.083 vs. 0.005, p=0.04). Compared to amyloid negative MCI,

amyloid positive MCI manifested significantly greater anxiety FxS (0.569 vs. 0.314, p=0.023), delusions FxS (0.138 vs. 0.004, p=0.001), and aberrant motor behaviors FxS (0.286 vs. 0.088, p=0.025). Compared to amyloid negative dementia subjects, amyloid positive dementia cases showed significantly lower apathy FxS (1.271 vs. 2.583, p=0.011), agitation FxS (0.619 vs. 1.708, p=0.003) and appetite changes (0.864 vs. 2.417, p=0.006), however these findings should be cautiously interpreted due to the very small sample size of the amyloid negative dementia group (N=25 vs. N=118 amyloid positive). No significant differences were seen in SMC. The full statistical models can be seen in **Supplementary Table 2**.

Cumulative incidence of NPS in follow-up:

No differences in the rates of de novo development of NPS were seen between amyloid positive and amyloid negative subjects in the NC, SMC or dementia groups. New onset delusions (13.4% vs. 2.2%, p<0.001), hallucinations (8.0% vs. 2.2%, p=0.007), anxiety (35.9% vs. 24.8%, p=0.014), apathy (38.4% vs. 21.8%, p<0.001), disinhibition (24.2% vs. 14.7%, p=0.014), irritability (45.5% vs. 32.7%, p=0.014), aberrant motor disturbances (18.1% vs. 9.2%, p=0.008**),** and appetite disturbances (33.5% vs. 20.9%, p=0.007) were significantly more common in amyloid positive vs. amyloid negative MCI **(Figure 1).** The full statistical models can be seen in **Supplemental Table 3**.

Predictors of amyloid status at baseline:

In our pooled stepwise backwards logistic regression model, anxiety [odds ratio (OR)=1.87, 95% confidence interval (95%CI) 1.17-3.02, p=0.018] and delusions (OR=7.01, 95%CI 1.25-132.05, p=0.04) were predictive of amyloid positivity after adjusting for age, sex, education, and *APOE4* in the pooled sample. When we analyzed the same relationship using the continuous FxS measure, delusions were a significant predictor (OR=2.22, 95%CI 1.18-6.23, p=0.012).

Among MCI participants the presence of anxiety (OR=1.77, 95%CI 1.03-3.09, p=0.04) at baseline was predictive of AD pathology. Using the continuous FxS measure revealed significant association between delusions and amyloid pathology (OR=5.8, 95%CI 1.69-98.36, p=0.001).

No significant differences were seen among the individual NC, SMC, or dementia groups.

Time to development of the five most frequent early NPS by amyloid status:

Here we focused on the five most common early NPS – apathy, anxiety, agitation, irritability and depression. Time to de novo development of these NPS in NC and SMC did not differ by amyloid status. After adjusting for age, education, and *APOE4* status, amyloid pathology in MCI was associated with faster emergence of agitation [hazard ratio (HR)=1.47, 95%CI 1.09-1.99, p=0.012], anxiety (HR=1.45, 95%CI 1.07-1.98, p=0.017), and apathy (HR=1.34, 95%CI 0.98-1.83, p<0.001) **(Figure 2)**.

Effect of baseline NPS on time to conversion in MCI:

Individually, adjusting for age, education and *APOE4* status, four of the five early NPS at baseline were significantly associated with time to conversion from MCI to dementia (agitation HR=2.28, 95%CI 1.64-3.17, p<0.001; depression HR=1.51, 95%CI 1.08-2.11, p=0.021; irritability HR=1.88, 95%CI 1.35-2.63, p<0.001; anxiety HR=1.39, 95%CI 0.96-2.02, p=0.083; apathy HR=2.69, 95%CI 1.92-3.78, p<0.001). Additionally, adjusting for amyloid status did not change the results (agitation HR=1.78, 95%CI 1.27-2.49, p=0.002; depression HR=1.44, 95%CI 1.03-2.02, p=0.04; irritability HR=1.61, 95%CI 1.15-2.25, p=0.009; anxiety HR=1.2, 95%CI 0.82-1.75, p=0.341; apathy HR=2.57, 95%CI 1.83-3.61, p<0.001).

When all five symptoms were entered simultaneously into a multivariable Cox regression model adjusting for age, education and *APOE4* status agitation and apathy remained significant (agitation HR=1.76, 95%CI 1.19-2.6, p=0.005; apathy HR=2.59, 95%CI 1.81-3.71, p<0.001). After additionally adjusting for baseline amyloid status apathy remained significant (agitation HR=1.46, 95%CI 0.99-2.15, p=0.057; apathy HR=2.48, 95%CI 1.73-3.56, p<0.001) **(Figure 3).**

Effect of de novo NPS on time to conversion in MCI:

Individually, adjusting for age, education and *APOE4* status, the emergence of agitation, depression, anxiety and apathy were associated with greater risk for conversion from MCI to dementia (agitation HR=2.17, 95%CI 1.47-3.2, p<0.001; depression HR=1.54, 95%CI 1.02-2.33, p=0.049; anxiety HR=2.17, 95%CI 1.47-3.2, p<0.001; apathy HR=2.33, 95%CI 1.57-3.45, p<0.001). Agitation, anxiety, and apathy remained significant when we also adjusted for amyloid status (agitation

HR=2.03, 95%CI 1.37-3, p=0.001; anxiety HR=2.03, 95%CI 1.37-3, p=0.001; apathy HR=2.42, 95%CI 1.65-3.57, p<0.001).

When all five symptoms were entered simultaneously into a multivariable Cox regression model adjusting for age, education and *APOE4* status, de novo agitation, irritability, and apathy were significant predictors of conversion from MCI to dementia (agitation HR=2.0, 95%CI 1.17-3.43, p=0.012; irritability HR=0.45, 95%CI 0.24-0.82, p=0.009; apathy HR=2.09, 95%CI 1.17-3.73, p=0.012). Additionally, adjusting for baseline amyloid status, did not change the results (agitation HR=1.91, 95%CI 1.12-3.28, p=0.018; irritability HR=0.47, 95%CI 0.26-0.86, p=0.014; apathy HR=2.42, 95%CI 1.39-4.23, p=0.002) **(Figure 4)**.

DISCUSSION

Here we investigated the relationship between amyloid burden and incidence and frequency of NPS across the spectrum from normal cognition to dementia. We hypothesized that amyloid pathology will associate with higher frequency of NPS across all disease stages, but we found that while the frequency of NPS is not significantly different between amyloid negative and positive diagnostic groups, some NPS might be more severe in those who are amyloid positive (Supplementary Table 2). This held true for aberrant motor behaviors among amyloid positive NC and MCI, as well as anxiety and delusions in amyloid positive MCI. Our findings in the MCI group is in line with those by Krell-Roesch et al., 2019, who found that compared to MCI and NC without amyloid burden, MCI with amyloid burden but not NC with amyloid burden had an increased risk of

having NPS[26]. Looking into whether neuropsychiatric symptoms carry prognostic value of Alzheimer's pathology we found anxiety to be predictive of brain amyloidosis in the pooled and MCI samples. Delusions carried additional predictive value in MCI.

In line with our hypothesis, a greater proportion of the amyloid positive MCI had emergence of delusions, hallucinations, anxiety, apathy, disinhibition, irritability, aberrant motor behaviors and appetite changes compared to amyloid negative MCI over the course of the study (Figure 1). These findings are as expected considering the prominence and progressive development of NPS over time during the course of the disease [6 7]. Our Cox proportional hazard regression model further demonstrated that participants with MCI due to AD develop apathy, anxiety, and agitation - three of the earliest and most pervasive NPS in the MCI stage, earlier compered to amyloid negative MCI (Figure 2) [6].

Contrary to our expectations we found that amyloid negative dementia subjects have higher frequency of apathy, agitation and appetite changes compared to the amyloid positive dementia group. One possible explanation is that previous studies on the prevalence of NPS in AD dementia have not included biomarker validation. This means that AD phenocopies with amnestic presentation were included as AD cases. ADNI is the first large scale observational study that included amyloid PET as a biomarker. Thus, for the first time we have the opportunity to investigate the frequency of NPS in biomarker validated AD dementia and compare that to AD phenocopies. What we find here suggests that these previous reports might have overestimated the true prevalence of some NPS

in AD dementia due to including amyloid negative AD phenocopies in the AD dementia group. An alternative explanation is that ADNI subjects who are amyloid negative have other neurodegenerative disorders such as frontotemporal dementia, dementia with Lewy bodies, argyrophilic grain disease, hippocampal sclerosis, etc. These other pathologic entities could also explain the increased frequencies of NPS.

The survival analyses looking into the effects of the five most frequent early NPS on time to conversion from MCI to dementia showed that four of the 5 early NPS symptoms – agitation, apathy, depression and irritability, were individually predictive of conversion from MCI to dementia as previously reported [10 13-19]. Of these agitation and apathy remained significant when adjusting for the presence of all five behaviors as previously reported [13 14 17].

When we focused on the predictive effects of newly emerging symptoms on future conversion from MCI to dementia, 4 of the 5 symptoms - agitation, depression, anxiety and apathy, were individually predictive. Agitation, irritability, and apathy proved to be the main drivers of this relationship when adjusting for the presence of all five behaviors.

Taken together our data seem to indicate that both prevalent agitation, and apathy, and incident agitation, irritability and apathy are predictive of faster functional decline and loss of independence among amyloid positive and negative MCI. Our findings regarding agitation seem to agree with those reported by Brodaty et al.[13] who also found that agitation was significantly associated with cognitive decline. Several studies have also concluded that apathy is a useful NPS

for identifying cognitive decline to AD[18 46]. Affective symptoms, including irritability, have also been previously reported in association with later cognitive decline to dementia. [47]

Contrary to our expectations and the observations of others [11 30] NPS were not associated with faster cognitive decline among ADNI's amyloid positive NC. We could hypothesize that both the sample size differences (N=275 in our study vs. N=1,587 in the large population-based study by Geda et al.[11]) as well as the different inclusion criteria used by these two studies (i.e., the mandatory lack of even perceived age-associated decline and the exclusion of individuals with baseline depression defined as GDS>5 in ADNI NC) and the fact that Geda et al. did not have information regarding brain amyloidosis, as potential drivers for these differences.

Several strengths and limitations of the ADNI study should be acknowledged. One of the strengths of this study is our cohort. ADNI is the premier longitudinal biomarker study in AD. ADNI employs unified subject assessment and PET scan collection protocols and meticulous data quality control across all study sites. Another strength of our study is the relatively large sample size and the inclusion of all diagnostic groups across the AD spectrum instead of just the symptomatic ones as it has been done in the past. Another strength of our paper is the use of neuropsychiatric data collection tools that are administered to the caregivers and not the subject themselves. One of the limitations of this study is that the NPI and NPI-Q use structured questions focused on the presence and severity of symptoms from the preceding month only, and therefore, intermittent

NPS that were not manifested by the subjects in the pre-specified timeframe were not captured. Another limitation to our study is that ADNI employs rigorous exclusion criteria typical of clinical trials and the study population and hence our study cohort might not be representative of the general population. Moreover, ADNI criteria excludes subjects with GDS greater than or equal to 5 and any primary psychiatric condition. These selection criteria undoubtedly have further influenced on our study and might limit the overall generalizability of our findings to the elderly population as a whole. Lastly, since the model selection and model building were done in the same dataset, our results are considered explanatory. Further studies are warranted to confirm our results.

In summary, we investigated the relationship between amyloid burden and occurrence of NPS in elderly with and without cognitive decline at baseline and over time. We found that amyloid pathology is a significant risk factor for future development of NPS in MCI, but not in the presymptomatic or at-risk stages of the disease. We also found that the presence of apathy, agitation, depression and irritability in MCI patients predict a more aggressive disease course regardless of the presence or absence of amyloid pathology.

References

1. Barthel H, Gertz HJ, Dresel S, et al. Cerebral amyloid-beta PET with florbetaben (18F) in patients with Alzheimer's disease and healthy controls: a multicentre phase 2 diagnostic study. *Lancet Neurol* 2011;10(5):424-35. doi: 10.1016/S1474-4422(11)70077-1
2. Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA* 2011;305(3):275-83. doi: 10.1001/jama.2010.2008
3. Vandenberghe R, Van Laere K, Ivanoiu A, et al. 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: a phase 2 trial. *Ann Neurol* 2010;68(3):319-29. doi: 10.1002/ana.22068
4. Dubois B, Hampel H, Feldman HH, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement* 2016;12(3):292-323. doi: 10.1016/j.jalz.2016.02.002
5. Sperling R, Mormino E, Johnson K. The evolution of preclinical Alzheimer's disease: implications for prevention trials. *Neuron* 2014;84(3):608-22. doi: 10.1016/j.neuron.2014.10.038
6. Apostolova LG, Cummings JL. Neuropsychiatric manifestations in mild cognitive impairment: a systematic review of the literature. *Dement Geriatr Cogn Disord* 2008;25(2):115-26. doi: 10.1159/000112509 [published Online First: 2007/12/19]
7. Mega MS, Cummings JL, Fiorello T, et al. The spectrum of behavioral changes in Alzheimer's disease. *Neurology* 1996;46(1):130-5.
8. Steinberg M, Shao H, Zandi P, et al. Point and 5-year period prevalence of neuropsychiatric symptoms in dementia: the Cache County Study. *Int J Geriatr Psychiatry* 2008;23(2):170-7. doi: 10.1002/gps.1858 [published Online First: 2007/07/04]
9. Lyketsos CG, Steinberg M, Tschanz JT, et al. Mental and behavioral disturbances in dementia: findings from the Cache County Study on Memory in Aging. *Am J Psychiatry* 2000;157(5):708-14.
10. Donovan NJ, Amariglio RE, Zoller AS, et al. Subjective cognitive concerns and neuropsychiatric predictors of progression to the early clinical stages of Alzheimer disease. *Am J Geriatr Psychiatry* 2014;22(12):1642-51. doi: 10.1016/j.jagp.2014.02.007
11. Geda YE, Roberts RO, Mielke MM, et al. Baseline neuropsychiatric symptoms and the risk of incident mild cognitive impairment: a population-based study. *Am J Psychiatry* 2014;171(5):572-81. doi: 10.1176/appi.ajp.2014.13060821
12. Zufferey V, Donati A, Popp J, et al. Neuroticism, depression, and anxiety traits exacerbate the state of cognitive impairment and hippocampal vulnerability to Alzheimer's disease. *Alzheimers Dement (Amst)* 2017;7:107-14. doi: 10.1016/j.dadm.2017.05.002 [published Online First: 2017/06/28]
13. Brodaty H, Heffernan M, Draper B, et al. Neuropsychiatric symptoms in older people with and without cognitive impairment. *J Alzheimers Dis* 2012;31(2):411-20. doi: 10.3233/JAD-2012-120169

14. Palmer K, Di Iulio F, Varsi AE, et al. Neuropsychiatric predictors of progression from amnestic-mild cognitive impairment to Alzheimer's disease: the role of depression and apathy. *J Alzheimers Dis* 2010;20(1):175-83. doi: 10.3233/JAD-2010-1352
15. Pink A, Stokin GB, Bartley MM, et al. Neuropsychiatric symptoms, APOE epsilon4, and the risk of incident dementia: a population-based study. *Neurology* 2015;84(9):935-43. doi: 10.1212/WNL.0000000000001307
16. Rosenberg PB, Mielke MM, Appleby BS, et al. The association of neuropsychiatric symptoms in MCI with incident dementia and Alzheimer disease. *Am J Geriatr Psychiatry* 2013;21(7):685-95. doi: 10.1016/j.jagp.2013.01.006
17. Somme J, Fernandez-Martinez M, Molano A, et al. Neuropsychiatric symptoms in amnestic mild cognitive impairment: increased risk and faster progression to dementia. *Curr Alzheimer Res* 2013;10(1):86-94.
18. Teng E, Lu PH, Cummings JL. Neuropsychiatric symptoms are associated with progression from mild cognitive impairment to Alzheimer's disease. *Dement Geriatr Cogn Disord* 2007;24(4):253-9. doi: 10.1159/000107100
19. Wadsworth LP, Lorius N, Donovan NJ, et al. Neuropsychiatric symptoms and global functional impairment along the Alzheimer's continuum. *Dement Geriatr Cogn Disord* 2012;34(2):96-111. doi: 000342119
20. Peters ME, Schwartz S, Han D, et al. Neuropsychiatric symptoms as predictors of progression to severe Alzheimer's dementia and death: the Cache County Dementia Progression Study. *Am J Psychiatry* 2015;172(5):460-5. doi: 10.1176/appi.ajp.2014.14040480 [published Online First: 2015/01/15]
21. Rabins PV, Schwartz S, Black BS, et al. Predictors of progression to severe Alzheimer's disease in an incidence sample. *Alzheimers Dement* 2013;9(2):204-7. doi: 10.1016/j.jalz.2012.01.003 [published Online First: 2012/11/06]
22. Fischer CE, Ismail Z, Schweizer TA. Impact of neuropsychiatric symptoms on caregiver burden in patients with Alzheimer's disease. *Neurodegenerative Disease Management* 2012;2:269-77.
23. Cepoiu-Martin M, Tam-Tham H, Patten S, et al. Predictors of long-term care placement in persons with dementia: a systematic review and meta-analysis. *Int J Geriatr Psychiatry* 2016;31(11):1151-71. doi: 10.1002/gps.4449 [published Online First: 2016/10/19]
24. Gaugler JE, Yu F, Krichbaum K, et al. Predictors of nursing home admission for persons with dementia. *Med Care* 2009;47(2):191-8. doi: 10.1097/MLR.0b013e31818457ce [published Online First: 2009/01/27]
25. Geda YE, Schneider LS, Gitlin LN, et al. Neuropsychiatric symptoms in Alzheimer's disease: past progress and anticipation of the future. *Alzheimers Dement* 2013;9(5):602-8. doi: 10.1016/j.jalz.2012.12.001 [published Online First: 2013/04/09]
26. Krell-Roesch J, Vassilaki M, Mielke MM, et al. Cortical beta-amyloid burden, neuropsychiatric symptoms, and cognitive status: the Mayo Clinic Study of Aging. *Transl Psychiatry* 2019;9(1):123. doi: 10.1038/s41398-019-0456-z [published Online First: 2019/03/30]

27. Marshall GA, Donovan NJ, Lorius N, et al. Apathy is associated with increased amyloid burden in mild cognitive impairment. *J Neuropsychiatry Clin Neurosci* 2013;25(4):302-7. doi: 10.1176/appi.neuropsych.12060156
28. Ramakers IH, Verhey FR, Scheltens P, et al. Anxiety is related to Alzheimer cerebrospinal fluid markers in subjects with mild cognitive impairment. *Psychol Med* 2013;43(5):911-20. doi: 10.1017/S0033291712001870
29. Bensamoun D, Guignard R, Furst AJ, et al. Associations between Neuropsychiatric Symptoms and Cerebral Amyloid Deposition in Cognitively Impaired Elderly People. *J Alzheimers Dis* 2016;49(2):387-98. doi: 10.3233/JAD-150181
30. Pietrzak RH, Lim YY, Neumeister A, et al. Amyloid-beta, anxiety, and cognitive decline in preclinical Alzheimer disease: a multicenter, prospective cohort study. *JAMA Psychiatry* 2015;72(3):284-91. doi: 10.1001/jamapsychiatry.2014.2476
31. Babulal GM, Ghoshal N, Head D, et al. Mood Changes in Cognitively Normal Older Adults are Linked to Alzheimer Disease Biomarker Levels. *Am J Geriatr Psychiatry* 2016;24(11):1095-104. doi: 10.1016/j.jagp.2016.04.004
32. Petersen RC, Aisen PS, Beckett LA, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. *Neurology* 2010;74(3):201-9. doi: 10.1212/WNL.0b013e3181cb3e25 [published Online First: 2010/01/01]
33. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34(7):939-44.
34. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12(3):189-98.
35. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 1993;43(11):2412-4.
36. Wechsler D. WMS-R: Wechsler Memory Scale-Revised manual. San Antonio, TX: Psychological Corp., Harcourt Brace Jovanovich 1987.
37. Saykin AJ, Wishart HA, Rabin LA, et al. Older adults with cognitive complaints show brain atrophy similar to that of amnestic MCI. *Neurology* 2006;67(5):834-42. doi: 10.1212/01.wnl.0000234032.77541.a2
38. Cummings JL. The Neuropsychiatric Inventory: assessing psychopathology in dementia patients. *Neurology* 1997;48(5 Suppl 6):S10-6.
39. Kaufer DI, Cummings JL, Ketchel P, et al. Validation of the NPI-Q, a brief clinical form of the Neuropsychiatric Inventory. *J Neuropsychiatry Clin Neurosci* 2000;12(2):233-9. [published Online First: 2000/09/23]
40. Jagust WJ, Bandy D, Chen K, et al. The Alzheimer's Disease Neuroimaging Initiative positron emission tomography core. *Alzheimers Dement* 2010;6(3):221-9. doi: 10.1016/j.jalz.2010.03.003
41. Fleisher AS, Chen K, Liu X, et al. Using positron emission tomography and florbetapir F18 to image cortical amyloid in patients with mild cognitive impairment or dementia due to Alzheimer disease. *Arch Neurol* 2011;68(11):1404-11. doi: 10.1001/archneurol.2011.150

42. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a pracxtical and powerful approach to multiple testing. *J Royal Stat Soc Series B* 1995;57:289-300.
43. Weiner MW, Veitch DP, Aisen PS, et al. Impact of the Alzheimer's Disease Neuroimaging Initiative, 2004 to 2014. *Alzheimers Dement* 2015;11(7):865-84. doi: 10.1016/j.jalz.2015.04.005 [published Online First: 2015/07/22]
44. Weiner MW, Veitch DP, Aisen PS, et al. 2014 Update of the Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception. *Alzheimers Dement* 2015;11(6):e1-120. doi: 10.1016/j.jalz.2014.11.001 [published Online First: 2015/06/16]
45. Weiner MW, Veitch DP, Aisen PS, et al. Recent publications from the Alzheimer's Disease Neuroimaging Initiative: Reviewing progress toward improved AD clinical trials. *Alzheimers Dement* 2017;13(4):e1-e85. doi: 10.1016/j.jalz.2016.11.007 [published Online First: 2017/03/28]
46. Starkstein SE, Jorge R, Mizrahi R, et al. A prospective longitudinal study of apathy in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2006;77(1):8-11. doi: 10.1136/jnnp.2005.069575 [published Online First: 2005/12/20]
47. Ismail Z, Gatchel J, Bateman DR, et al. Affective and emotional dysregulation as pre-dementia risk markers: exploring the mild behavioral impairment symptoms of depression, anxiety, irritability, and euphoria - CORRIGENDUM. *Int Psychogeriatr* 2019;31(1):157. doi: 10.1017/S1041610218000637 [published Online First: 2018/11/22]

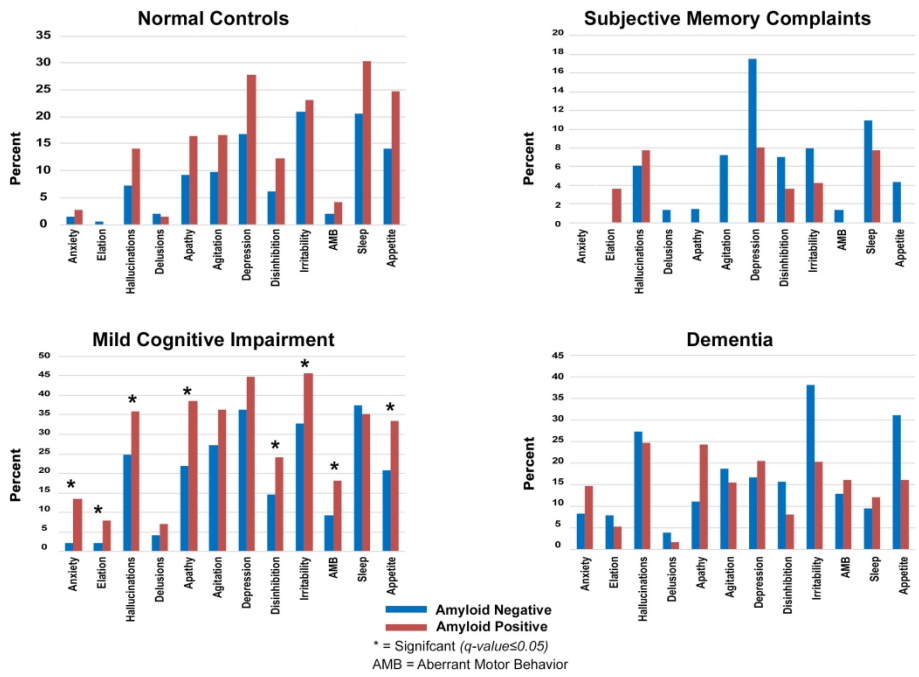
FIGURE LEGENDS

Figure 1: De novo emergence of NPS by diagnosis and amyloid status. NOTE: y-axis vary by diagnosis

Figure 2: Survival curves showing time to emergence of the five most frequent early NPS in MCI by amyloid status

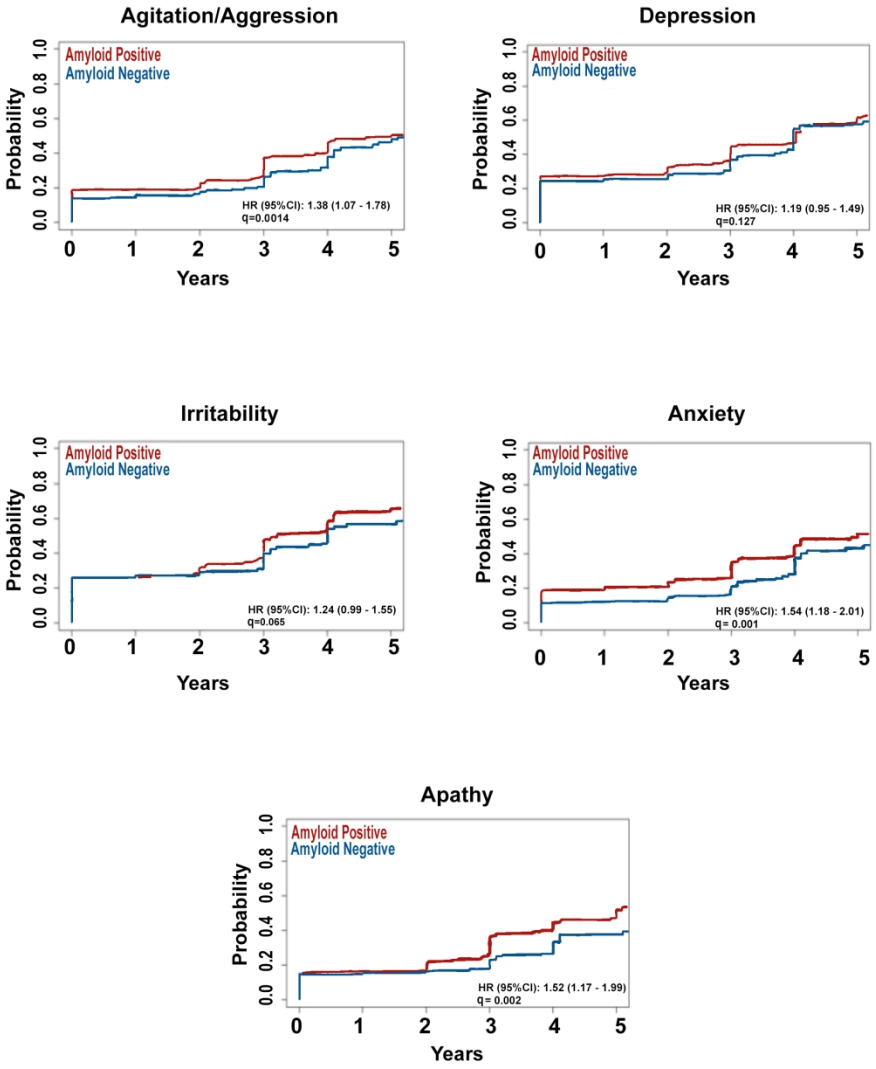
Figure 3: Survival curves showing the effect of the presence of the five most frequent early NPS on time to conversion from MCI to dementia

Figure 4: Survival curves showing the effect of de novo development of the five most frequent early NPS on time to conversion from MCI to dementia



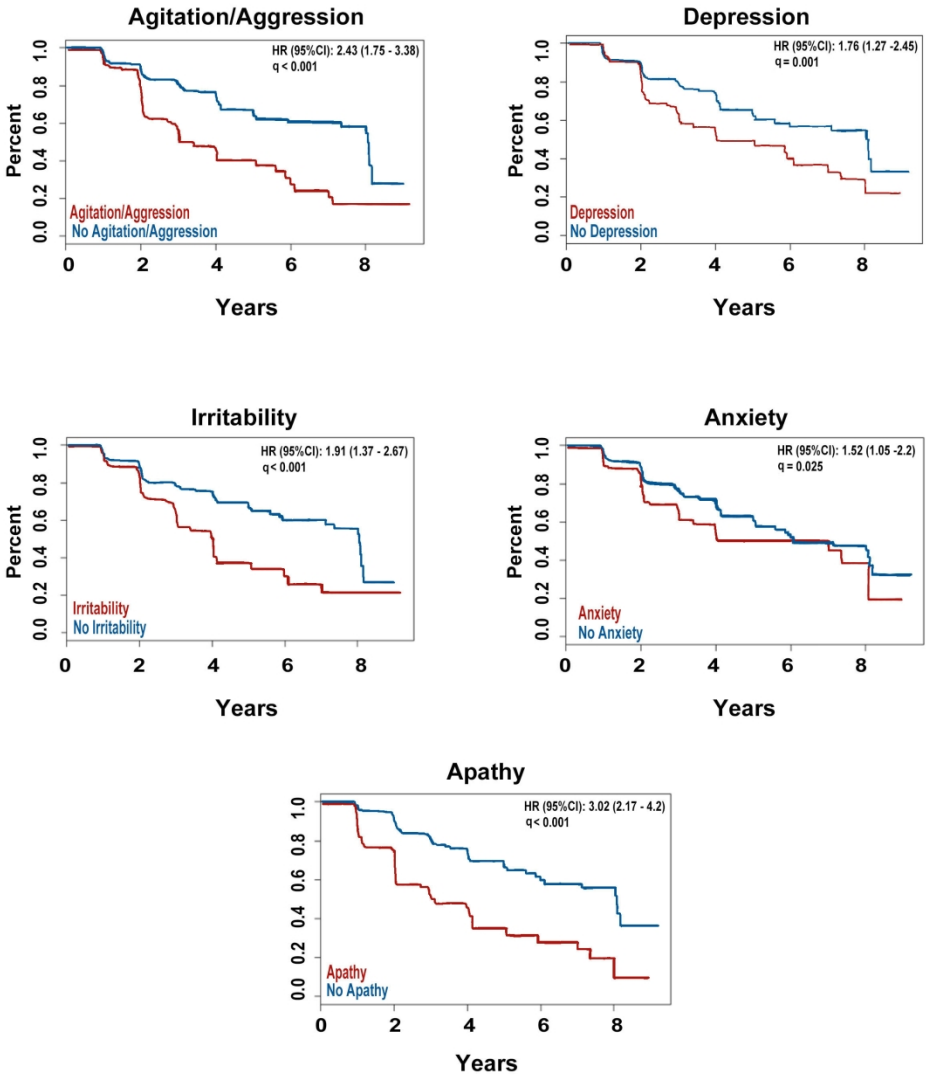
De novo emergence of NPS by diagnosis and amyloid status. NOTE: y-axis vary by diagnosis

254x172mm (300 x 300 DPI)



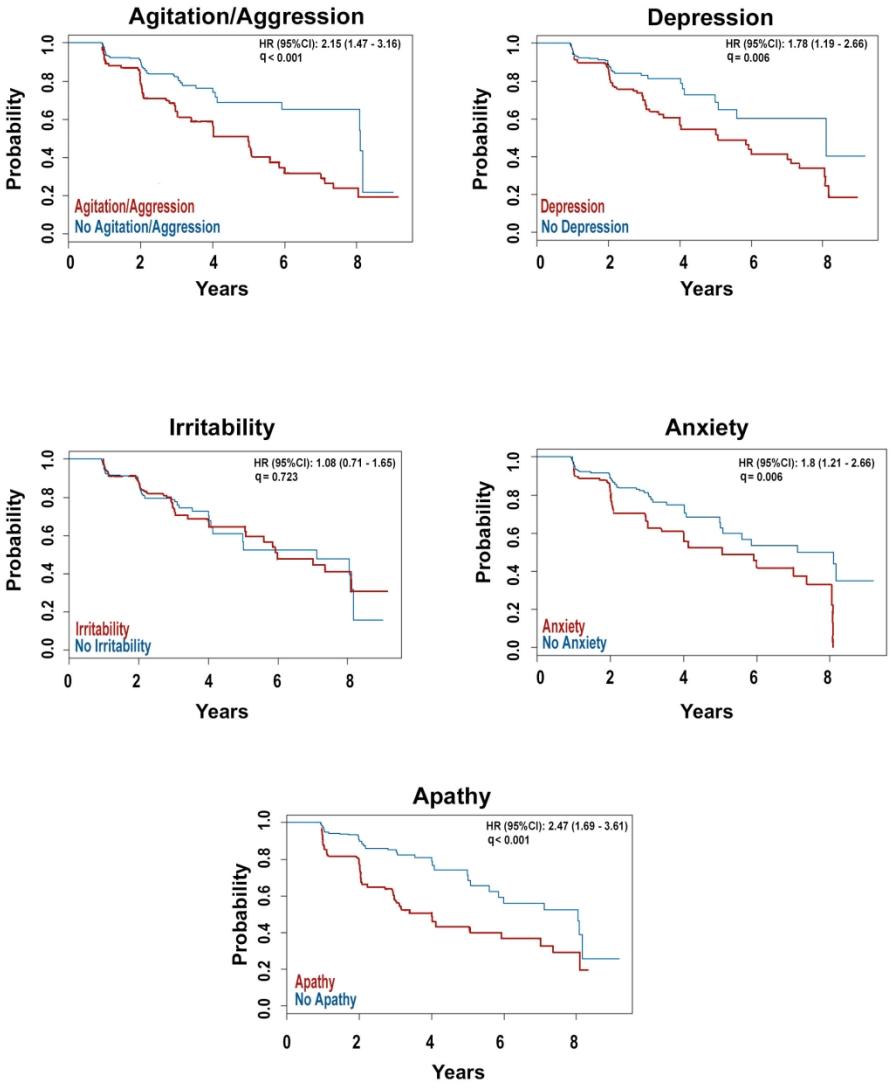
Survival curves showing time to emergence of the five most frequent early NPS in MCI by amyloid status

203x254mm (300 x 300 DPI)



Survival curves showing the effect of the presence of the five most frequent early NPS on time to conversion from MCI to dementia

203x254mm (300 x 300 DPI)



Survival curves showing the effect of de novo development of the five most frequent early NPS on time to conversion from MCI to dementia

203x254mm (300 x 300 DPI)

Supplemental Table 1

Variables		NC (N=275)				SMC (N=100)				MCI (N=559)				DEMENTIA (N=143)			
		Amyloid- (N=202)	Amyloid+ (N=73)	OR	q-value	Amyloid- (N=72)	Amyloid+ (N=28)	OR	q-value	Amyloid- (N=269)	Amyloid+ (N=290)	OR	q-value	Amyloid- (N=25)	Amyloid+ (N=118)	OR	q-value
Anxiety	**Yes**	10 (5)	2 (2.7)	0.54 (0.12, 2.52)	NS	6 (8.3)	2 (7.1)	0.85 (0.16, 4.49)	NS	31 (11.5)	55 (19)	1.8 (1.12, 2.9)	NS	3 (12)	33 (28)	2.85 (0.8, 10.16)	NS
Elation	**Yes**	0 (0)	0 (0)	∞	NS	0 (0)	0 (0)	∞	NS	9 (3.3)	7 (2.4)	0.71 (0.26, 1.93)	NS	0 (0)	2 (1.7)	∞	NS
Hallucinations	**Yes**	1 (0.5)	0 (0)	∞	NS	0 (0)	0 (0)	∞	NS	1 (0.4)	3 (1)	2.8 (0.29, 27.08)	NS	0 (0)	6 (5.1)	∞	NS
Delusions	**Yes**	0 (0)	0 (0)	∞	NS	0 (0)	0 (0)	∞	NS	0 (0)	7 (2.4)	∞	NS	1 (4)	9 (7.6)	1.98 (0.24, 16.38)	NS
Apathy	**Yes**	6 (3)	0 (0)	∞	NS	4 (5.6)	1 (3.6)	0.63 (0.07, 5.9)	NS	40 (14.9)	45 (15.5)	1.05 (0.66, 1.67)	NS	16 (64)	44 (37.3)	0.33 (0.13, 0.81)	NS
Agitation	**Yes**	9 (4.5)	1 (1.4)	0.3 (0.04, 2.41)	NS	3 (4.2)	3 (10.7)	2.76 (0.52, 14.58)	NS	38 (14.1)	55 (19)	1.42 (0.9, 2.23)	NS	9 (36)	34 (28.8)	0.72 (0.29, 1.79)	NS
Depression	**Yes**	17 (8.4)	1 (1.4)	0.15 (0.02, 1.15)	NS	9 (12.5)	3 (10.7)	0.84 (0.21, 3.36)	NS	65 (24.2)	78 (26.9)	1.15 (0.79, 1.68)	NS	7 (28)	45 (38.1)	1.59 (0.62, 4.11)	NS
Disinhibition	**Yes**	5 (2.5)	0 (0)	∞	NS	1 (1.4)	0 (0)	∞	NS	24 (8.9)	30 (10.3)	1.18 (0.67, 2.07)	NS	6 (24)	20 (16.9)	0.65 (0.23, 1.83)	NS
Irritability	**Yes**	20 (9.9)	4 (5.5)	0.53 (0.17, 1.61)	NS	9 (12.5)	4 (14.3)	1.17 (0.33, 4.16)	NS	70 (26)	76 (26.2)	1.01 (0.69, 1.47)	NS	4 (16)	39 (33.1)	2.59 (0.83, 8.07)	NS
Aberrant motor behavior	**Yes**	2 (1)	0 (0)	∞	NS	1 (1.4)	0 (0)	∞	NS	7 (2.6)	13 (4.5)	1.76 (0.69, 4.48)	NS	2 (8)	19 (16.1)	2.21 (0.48, 10.17)	NS
Sleep disturbance	**Yes**	22 (10.9)	7 (9.6)	0.87 (0.36, 2.13)	NS	17 (23.6)	2 (7.1)	0.25 (0.05, 1.16)	NS	55 (20.4)	59 (20.3)	0.99 (0.66, 1.49)	NS	4 (16)	19 (16.1)	1.01 (0.31, 3.28)	NS
Appetite changes	**Yes**	3 (1.5)	0 (0)	∞	NS	3 (4.2)	1 (3.6)	0.85 (0.08, 8.53)	NS	25 (9.3)	21 (7.2)	0.76 (0.41, 1.39)	NS	9 (36)	25 (21.2)	0.48 (0.19, 1.21)	NS

Supplemental Table 2												
Variables	**NC (N=275)**			**SMC (N=100)**			**MCI (N=559)**			**DEMENTIA (N=143)**		
	Amyloid- (N=202)	Amyloid+ (N=73)	q-value	Amyloid- (N=72)	Amyloid+ (N=28)	q-value	Amyloid- (N=269)	Amyloid+ (N=290)	q-value	Amyloid- (N=25)	Amyloid+ (N=118)	q-value
Anxiety	0.11 (0.59)	0.10 (0.59)	NS	0.21 (1.01)	0.18 (0.77)	NS	0.31 (1.14)	0.57 (1.44)	**0.023**	0.38 (1.14)	0.70 (1.39)	NS
Elation	0 (0)	0 (0)	∞	0 (0)	0 (0)	∞	0.08 (0.51)	0.09 (0.67)	NS	0 (0)	0.08 (0.74)	NS
Hallucinations	0 (0)	0 (0)	∞	0 (0)	0 (0)	∞	0.02 (0.26)	0.07 (0.59)	NS	0 (0)	0.07 (0.31)	NS
Delusions	0 (0)	0 (0)	∞	0 (0)	0 (0)	∞	0.004 (0.06)	0.14 (0.64)	**0.001**	0.13 (0.61)	0.36 (1.45)	NS
Apathy	0.11 (0.89)	0.24 (1.19)	NS	0.14 (0.59)	0.11 (0.57)	NS	0.50 (1.61)	0.63 (1.73)	NS	2.58 (2.87)	1.27 (2.14)	**0.011**
Agitation	0.14 (0.95)	0.10 (0.42)	NS	0.06 (0.29)	0.18 (0.61)	NS	0.39 (1.11)	0.56 (1.35)	NS	1.71 (2.85)	0.62 (1.27)	**0.003**
Depression	0.23 (0.92)	0.18 (0.83)	NS	0.24 (0.83)	0.11 (0.32)	NS	0.64 (1.41)	0.72 (1.38)	NS	0.67 (1.24)	0.80 (1.27)	NS
Disinhibition	0.04 (0.21)	0.13 (0.75)	NS	0.03 (0.24)	0 (0)	NS	0.25 (1.02)	0.31 (1.11)	NS	0.71 (1.37)	0.32 (1.04)	NS
Irritability	0.21 (0.88)	0.29 (0.86)	NS	0.26 (0.79)	0.25 (0.65)	NS	0.78 (1.78)	0.75 (1.54)	NS	0.63 (1.61)	1.02 (2.063)	NS
Aberrant motor behavior	0.01 (0.07)	0.08 (0.52)	NS	0.04 (0.35)	0 (0)	NS	0.09 (0.64)	0.29 (1.28)	**0.025**	0.29 (1.0)	0.59 (1.62)	NS
Sleep disturbance	0.38 (1.29)	0.49 (1.28)	NS	0.57 (1.23)	0.25 (0.93)	NS	0.85 (1.94)	0.99 (2.26)	NS	0.46 (1.14)	0.59 (1.58)	NS
Appetite changes	0.10 (0.71)	0.24 (1.22)	NS	0.21 (1.17)	0.04 (0.19)	NS	0.29 (1.22)	0.51 (1.70)	0.084	2.42 (3.86)	0.86 (2.1)	**0.006**

Supplemental Table 3

Variables		NC (N=275)			SMC (N=100)			MCI (N=559)			DEMENTIA (N=143)		
		Amyloid- (N=202)	Amyloid+ (N=73)	q-value	Amyloid- (N=72)	Amyloid+ (N=28)	q-value	Amyloid- (N=269)	Amyloid+ (N=290)	q-value	Amyloid- (N=25)	Amyloid+ (N=118)	q-value
Anxiety	**Yes**	3 (1.5)	2 (2.7)	NS	0 (0)	0 (0)	NS	6 (2.2)	38 (13.4)	**< 0.001**	2 (8.3)	16 (14.7)	NS
Elation	**Yes**	1 (0.5)	0 (0)	NS	0 (0)	1 (3.6)	NS	6 (2.2)	23 (8)	**0.007**	2 (8)	6 (5.4)	NS
Hallucinations	**Yes**	14 (7.3)	10 (14.1)	NS	4 (6.1)	2 (7.7)	NS	59 (24.8)	84 (35.9)	**0.014**	6 (27.3)	21 (24.7)	NS
Delusions	**Yes**	4 (2)	1 (1.4)	NS	1 (1.4)	0 (0)	NS	11 (4.2)	20 (7.1)	NS	1 (4)	2 (1.7)	NS
Apathy	**Yes**	18 (9.2)	12 (16.4)	NS	1 (1.5)	0 (0)	NS	50 (21.8)	94 (38.4)	**< 0.001**	1 (11.1)	18 (24.3)	NS
Agitation	**Yes**	19 (9.8)	12 (16.7)	NS	5 (7.2)	0 (0)	NS	63 (27.3)	85 (36.2)	0.063	3 (18.8)	13 (15.5)	NS
Depression	**Yes**	31 (16.8)	20 (27.8)	NS	11 (17.5)	2 (8)	NS	74 (36.3)	95 (44.8)	NS	3 (16.7)	15 (20.5)	NS
Disinhibition	**Yes**	12 (6.1)	9 (12.3)	NS	5 (7)	1 (3.6)	NS	36 (14.7)	63 (24.2)	**0.014**	3 (15.8)	8 (8.2)	NS
Irritability	**Yes**	38 (20.9)	16 (23.2)	NS	5 (7.9)	1 (4.2)	NS	65 (32.7)	97 (45.5)	**0.014**	8 (38.1)	16 (20.3)	NS
Aberrant motor behavior	**Yes**	4 (2)	3 (4.1)	NS	1 (1.4)	0 (0)	NS	24 (9.2)	50 (18.1)	**0.00**8	3 (13)	16 (16.2)	NS
Sleep disturbance	**Yes**	37 (20.6)	20 (30.3)	NS	6 (10.9)	2 (7.7)	NS	80 (37.4)	81 (35.2)	NS	2 (9.5)	12 (12.1)	NS
Appetite changes	**Yes**	28 (14.1)	18 (24.7)	NS	3 (4.3)	0 (0)	NS	51 (20.9)	90 (33.5)	**0.007**	5 (31.2)	15 (16.1)	NS

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	8-9	
Objectives	3	State specific objectives, including any prespecified hypotheses	9	
Methods				
Study design	4	Present key elements of study design early in the paper	11-14	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	11-12	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	11-12	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	11-12	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-12	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11-12	
Bias	9	Describe any efforts to address potential sources of bias	14	
Study size	10	Explain how the study size was arrived at	11-12	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	14-15
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	14-15
		(*b*) Describe any methods used to examine subgroups and interactions	14-15
		(*c*) Explain how missing data were addressed	14-15
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	16
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	16
		(b) Indicate number of participants with missing data for each variable of interest	16
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	16
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	18-21
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	17-21
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	20-22
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	24
Generalisability	21	Discuss the generalisability (external validity) of the study results	24
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	4

Text

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.