

Anxiety is related to Alzheimer cerebrospinal fluid markers in subjects with mild cognitive impairment

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Background. Anxiety, apathy and depression are common in subjects with mild cognitive impairment (MCI) and may herald Alzheimer's disease (AD). We investigated whether these symptoms correlated with cerebrospinal fluid (CSF) markers for AD in subjects with MCI.

Method. Subjects with MCI ($n=268$) were selected from the 'Development of screening guidelines and criteria for pre-dementia Alzheimer's disease' (DESCRIPA) and Alzheimer's Disease Neuroimaging Initiative (ADNI) studies. We measured amyloid $\beta_{(1-42)}$ protein ($A\beta_{42}$) and total tau (t-tau) in CSF. Neuropsychiatric symptoms were measured with the Neuropsychiatric Inventory.

Results. Depressive symptoms were reported by 55 subjects (21%), anxiety by 35 subjects (13%) and apathy by 49 subjects (18%). The presence of anxiety was associated with abnormal CSF $A\beta_{42}$ [odds ratio (OR) 2.3, 95% confidence interval (CI) 1.6–3.3] and t-tau (OR 2.6, 95% CI 1.9–3.6) concentrations and with the combination of abnormal concentrations of both $A\beta_{42}$ and t-tau (OR 3.1, 95% CI 2.0–4.7). The presence of agitation and irritability was associated with abnormal concentrations of $A\beta_{42}$ (agitation: OR 1.6, 95% CI 1.1–2.3; irritability: OR 2.2, 95% CI 1.5–3.3). Symptoms of depression and apathy were not related to any of the CSF markers.

Conclusions. In subjects with MCI, symptoms of anxiety, agitation and irritability may reflect underlying AD pathology, whereas symptoms of depression and apathy do not.

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Key words: Alzheimer's disease, anxiety, apathy, biomarkers, cerebrospinal fluid, depression, mild cognitive impairment, neuropsychiatric symptoms.

Background

Neuropsychiatric symptoms are common in subjects with mild cognitive impairment (MCI) and may be associated with an increased risk of Alzheimer's disease (AD)-type dementia in these subjects (Modrego & Ferrandez, 2004; Teng *et al.* 2007). To further clarify

the relationship between neuropsychiatric symptoms and AD pathology in subjects with MCI, we investigated the relationship between neuropsychiatric symptoms and key *in vivo* biomarkers for AD in the cerebrospinal fluid (CSF).

For neuropsychiatric symptoms, we selected depression, anxiety and apathy. These symptoms are among the most frequent neuropsychiatric symptoms among subjects with MCI (Lyketsos *et al.* 2002; Apostolova & Cummings, 2007; Geda *et al.* 2008). Prospective studies have shown that depression, anxiety and apathy are associated with an increased risk of AD (Modrego & Ferrandez, 2004; Teng *et al.* 2007), but

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other studies have not found such an association (Visser *et al.* 2000; Rozzini *et al.* 2005; Ramakers *et al.* 2010). In addition, we investigated the relationship between other neuropsychiatric symptoms present in more than 10% of the subjects and AD CSF markers.

As *in vivo* biomarkers for AD, we used the concentrations of amyloid $\beta_{(1-42)}$ protein (A β 42) and total tau (t-tau) in the CSF (Sunderland *et al.* 2003; Hansson *et al.* 2006; DeKosky, 2008). These CSF markers correlate with plaque load and tangles, respectively, in neuropathological studies (Buerger *et al.* 2006; Tapiola *et al.* 2009). Furthermore, these markers are frequently abnormal in subjects with MCI (Visser *et al.* 2009) and strongly predict conversion to AD-type dementia in subjects with MCI (Hansson *et al.* 2006). The relationship between a CSF biomarker profile suggestive of AD-type dementia and neuropsychiatric symptoms in subjects with MCI has never before been investigated. We tested this association in two independent cohorts, the 'Development of screening guidelines and criteria for pre-dementia Alzheimer's disease' (DESCRIPA) and Alzheimer's Disease Neuroimaging Initiative (ADNI) studies.

Method

Subjects

Subjects were selected from among the subjects included in the DESCRIPA and ADNI studies. The DESCRIPA study is a multi-center, prospective cohort study of 881 non-demented subjects selected from 20 out-patient memory clinics in 11 European countries (Visser *et al.* 2008). Inclusion criteria were an age of 55 years or older and being a new referral for the evaluation of cognitive complaints. Exclusion criteria were a diagnosis of dementia according to Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria (APA, 1994) at baseline and any somatic, psychiatric or neurological disorders that may have caused the cognitive impairment, as described in detail elsewhere (Visser *et al.* 2008). The data collection protocols varied among the centers. For the present study, we selected subjects with MCI from six centers ($n=240$) at which CSF was collected and the Neuropsychiatric Inventory (NPI) was performed at baseline. MCI was defined as a Clinical Dementia Rating Scale (CDR) global score of 0.5 and no dementia at baseline. Subjects who had available CSF and NPI data ($n=74$) had a significantly lower Mini Mental State Examination (MMSE) score (26.1 *v.* 27.3) and had fewer years of education (10.3 *v.* 12.0 years) compared with those for whom CSF or an NPI score was not available. Age was similar in both groups. Of these 74 subjects, 45 had amnesic MCI, defined as a memory

score of -1.5 S.D. below the score of healthy controls (Visser *et al.* 2008).

The ADNI study is a large, multicenter, longitudinal imaging study (Mueller *et al.* 2005). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations. Details about the ADNI cohort and the progress to date are described elsewhere (Shaw *et al.* 2009; Aisen *et al.* 2010; Trojanowski *et al.* 2010; Weiner *et al.* 2010). The ADNI included healthy control subjects, subjects with amnesic MCI and subjects with AD. In the present study, we used subjects with MCI, defined as an age of 55 years or older, a CDR memory box score of 0.5, impaired memory scores and no dementia. Exclusion criteria were any serious neurological disease other than possible AD, any history of brain lesions or head trauma, or psychoactive medication use. We selected subjects from whom CSF was collected and for whom the NPI was scored at baseline ($n=193$). Subjects with available CSF and NPI data were similar with respect to age, gender distribution, MMSE score and number of years of education to subjects for whom these data were not available. In both studies, the local medical ethical committee in each participating center approved the study. Subjects were asked to provide written informed consent.

Baseline assessment

At baseline, all subjects underwent standardized physical and neurological examinations, assessment of global cognitive functioning using the MMSE (Folstein *et al.* 1975) and neuropsychological assessments (Visser *et al.* 2008; Aisen *et al.* 2010).

NPI

In the DESCRIPA cohort, the presence of neuropsychiatric symptoms was measured with the full NPI (Cummings *et al.* 1994). The full NPI is a widely used informant-based inventory that rates the presence or absence of 12 neuropsychiatric domains. In addition, the frequency and severity of the symptoms present was rated. In the ADNI study, the informant-based NPI Questionnaire (NPI-Q) was used. The NPI-Q also rates the presence of the same neuropsychiatric symptoms. Unlike the full NPI, the NPI-Q only rates the severity of the symptoms. This implicates that no frequency scores were available from the ADNI cohort. Therefore, we dichotomized neuropsychiatric symptoms as present (1) or absent (0), which allows combining the scores from both scales. For

the investigation of the relationship between CSF biomarkers suggestive of AD and neuropsychiatric symptoms, we selected the key neuropsychiatric domains depression, anxiety and apathy. Secondary analyses were performed with neuropsychiatric domains for which prevalence was more than 10% in the pooled population: agitation, irritability, sleep and night-time behavior, and appetite and eating change.

CSF collection, storage and analysis

CSF was collected via a lumbar puncture. The procedure for the collection and analysis of CSF in the DESCRIPA study has been described elsewhere (Visser *et al.* 2009). In short, with a few exceptions, samples from the DESCRIPA study were collected and stored in polypropylene tubes (92%). Samples were centrifuged after collection and stored at -80°C until analysis. All CSF analyses were performed at the end of the study, using the same batch of reagents, at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. $A\beta_{1-42}$ and t-tau levels were measured using commercially available single-parameter enzyme-linked immunosorbent assay (ELISA) methods (Innotest[®] β -amyloid₁₋₄₂ and Innotest[®] hTAU-Ag, respectively; Innogenetics, Belgium). The intra-assay coefficient of variation (CV) is less than 5% ($n=501$) for the $A\beta_{1-42}$ ELISA method (Andreasen *et al.* 1999) and 4.6% ($n=127$) for the t-tau ELISA method (Olsson *et al.* 2005).

The CSF procedures in the ADNI study have been described in detail elsewhere (Shaw *et al.* 2009). Samples were collected and stored in polypropylene tubes and frozen. At the University of Pennsylvania ADNI Biomarker Core samples were defrosted, aliquoted and stored at -80°C . $A\beta_{42}$ and t-tau levels were measured using the multiplex xMAP Luminex platform (Luminex Corp., USA) and the INNO-BIA AlzBio3 kit (Innogenetics, Belgium), as described previously in detail (Olsson *et al.* 2005). The intra-assay CV for the Luminex technique is 2.0% ($n=141$) for $A\beta_{1-42}$ and 3.2% ($n=127$) for t-tau (Olsson *et al.* 2005), which is comparable with the ELISA methods.

CSF outcome measures

We dichotomized scores for $A\beta_{42}$ and t-tau as normal or abnormal based on published data. In the DESCRIPA study, concentrations below 500 ng/l for $A\beta_{42}$ (Sjogren *et al.* 2001) and above 320 ng/l for t-tau were classified as abnormal (Mattsson *et al.* 2009). In the ADNI study, $A\beta_{42}$ concentrations below 192 ng/l and t-tau concentrations above 93 ng/l were classified as abnormal (Shaw *et al.* 2009). Differences in cut-off scores were caused by the use of different platforms

for $A\beta_{42}$ and t-tau measurements in DESCRIPA (ELISA) and ADNI (xMAP). Although the absolute values of $A\beta_{42}$ and t-tau differ between the assays, the values are highly correlated (Reijn *et al.* 2007; Shaw *et al.* 2009; Fagan *et al.* 2011). Moreover, a direct comparison of the two platforms showed that each platform identified individuals with underlying amyloid plaque pathology equally well (Fagan *et al.* 2011).

Statistics

Statistical analyses were performed using SPSS version 11 for Mac OS X (SPSS Inc., USA). Analyses were performed in the pooled cohort using generalized linear models with corrections for age, center, use of polypropylene tubes, and whether samples had been defrosted before analysis. In a separate model, we tested the interaction between cohort (DESCRIPA *v.* ADNI) and CSF marker to determine whether the association between neuropsychiatric symptoms and CSF markers varied between the studies. Secondary analyses were performed in each cohort separately with adjustment for age, center, use of polypropylene tubes and whether samples had been defrosted before analysis.

Results

Subject characteristics

The baseline characteristics are presented in Table 1. A total of 157 subjects (59%) had one or more neuropsychiatric symptoms.

Relationship between depression, anxiety and apathy and CSF AD markers

In the pooled sample, $A\beta_{42}$ and t-tau concentrations were associated with symptoms of anxiety. Subjects with abnormal concentrations of $A\beta_{42}$ had more often symptoms of anxiety than subjects with normal concentrations [16% *v.* 8%, odds ratio (OR) 2.3, 95% confidence interval (CI) 1.6–3.3]. Subjects with abnormal concentrations of t-tau had more often symptoms of anxiety than subjects with normal concentrations (18% *v.* 8%, OR 2.6, 95% CI 1.9–3.6). Subjects who had abnormal concentrations of both $A\beta_{42}$ and t-tau had the highest prevalence of anxiety (21% *v.* 8%, OR 3.1, 95% CI 2.0–4.7, $p < 0.001$). In the pooled cohort, none of the CSF markers was related to the presence of depression and apathy (Table 2). Results were similar after correction for gender.

Similar findings were also found in the separate cohorts, except for the presence of apathy, which was significantly more likely to be present in subjects with normal $A\beta_{42}$ concentrations in the DESCRIPA cohort

Table 1. Subject characteristics

	Total	DESCRIPA	ADNI
Subjects, <i>n</i>	267	74	193
Mean age, years (s.d.)	73.1 (7.9)	69.8 (8.1)	74.4 (7.5)
Gender, % female	38	50	33
Mean education, years (s.d.)	14.3 (4.0)	10.3 (3.4)	15.8 (3.0)
Mean MMSE score (s.d.)	26.7 (2.1)	26.1 (2.8)	26.9 (1.8)
Apolipoprotein ε4 allele, %	54	55	53
Delusions, <i>n</i> (%)	4 (2)	3 (4)	1 (<1)
Hallucinations, <i>n</i> (%)	3 (1)	3 (4)	0 (0)
Agitation, <i>n</i> (%)	47 (18)	9 (12)	38 (20)
Depression, <i>n</i> (%)	55 (21)	16 (22)	39 (20)
Anxiety, <i>n</i> (%)	35 (13)	9 (12)	26 (13)
Euphoria, <i>n</i> (%)	6 (2)	0 (0)	6 (3)
Apathy, <i>n</i> (%)	49 (18)	21 (29)	28 (15)
Disinhibition, <i>n</i> (%)	17 (6)	3 (4)	14 (7)
Irritability, <i>n</i> (%)	74 (28)	20 (27)	54 (28)
Aberrant motor behavior, <i>n</i> (%)	15 (6)	3 (4)	12 (6)
Sleep and night-time behavior, <i>n</i> (%)	38 (14)	18 (24)	20 (10)
Appetite and eating changes, <i>n</i> (%)	30 (11)	10 (14)	20 (10)
Mean Aβ42, ng/l (s.d.)	–	500.7 (224.4) ^a	163.6 (55.1) ^b
Mean t-tau, ng/l (s.d.)	–	522.6 (383.5) ^a	103.3 (61.1) ^b
Aβ42 abnormal, <i>n</i> (%)	188 (70)	44 (59)	143 (74)
t-tau abnormal, <i>n</i> (%)	134 (50)	47 (64)	86 (45)
Aβ42 and t-tau abnormal, <i>n</i> (%)	113 (42)	29 (39)	83 (43)

DESCRIPA, Development of screening guidelines and criteria for pre-dementia Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; s.d., standard deviation; MMSE, Mini Mental State Examination; Aβ42, amyloid β_(1–42) protein, t-tau, total tau.

^a Concentrations based on enzyme-linked immunosorbent assay.

^b Concentrations based on xMAP Luminex.

Table 2. Relationship between the presence of neuropsychiatric symptoms and abnormal AD cerebrospinal fluid markers

	Depression	Anxiety	Apathy	Irritability	Agitation
Aβ42 abnormal					
Combined	1.2 (0.7–1.9)	2.3 (1.6–3.3)**	0.7 (0.4–1.2)	2.2 (1.5–3.3)**	1.6 (1.1–2.3)*
DESCRIPA	0.8 (0.2–2.4)	2.7 (0.9–8.3)	0.4 (0.2–0.9)*	3.6 (1.2–10.7)*	2.4 (0.5–12.1)
ADNI	1.5 (0.6–3.5)	2.1 (0.7–6.4)	1.1 (0.4–2.7)	1.8 (0.8–3.9)	1.4 (0.6–3.3)
t-tau abnormal					
Combined	1.2 (0.9–1.6)	2.6 (1.9–3.6)**	1.0 (0.7–1.6)	1.3 (0.8–2.2)	1.1 (0.7–1.5)
DESCRIPA	0.9 (0.4–2.1)	5.9 (1.3–25.9)*	1.3 (0.4–4.5)	0.7 (0.3–1.7)	0.5 (0.3–0.8)*
ADNI	1.4 (0.7–2.8)	2.3 (0.97–5.3)	0.9 (0.4–2.1)	1.7 (0.9–3.2)	1.3 (0.6–2.7)
t-tau and Aβ42 abnormal					
Combined	1.3 (0.96–1.7)	3.1 (2.0–4.7)**	0.9 (0.6–1.2)	1.3 (0.8–2.2)	1.1 (0.7–1.5)
DESCRIPA	0.9 (0.4–2.0)	7.3 (3.4–15.3)**	0.9 (0.4–2.2)	1.4 (0.4–4.8)	0.4 (0.1–1.4)
ADNI	1.5 (0.7–3.0)	2.5 (1.1–5.8)*	0.9 (0.4–1.9)	1.7 (0.9–3.2)	1.3 (0.6–2.6)

Data are given as odds ratio (95% confidence interval).

AD, Alzheimer's disease; Aβ42, amyloid β_(1–42) protein; DESCRIPA, Development of screening guidelines and criteria for pre-dementia Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; t-tau, total tau.

* *p* < 0.05, ** *p* < 0.001.

(OR 0.4, 95% CI 0.2–0.9; Table 2). Also the interaction analysis showed no difference in the relationship of neuropsychiatric symptoms and CSF markers between the cohorts.

After the exclusion of 29 subjects with non-amnesic MCI from the DESCRIPA cohort, the findings for the pooled cohort and for the DESCRIPA cohort remained essentially the same. Of the 74 subjects of the DESCRIPA cohort with a CDR global score of 0.5, 61 subjects had a CDR memory score of 0.5. After the exclusion of the 13 subjects with a CDR memory score of 1.0 from the DESCRIPA cohort, the findings for the DESCRIPA cohort remained essentially the same.

Relationship between other common neuropsychiatric symptoms and CSF AD markers

Agitation, irritability, sleep problems and eating problems were present in more than 10% of the subjects. In the pooled sample, subjects with abnormal CSF A β 42 concentrations had more often symptoms of agitation and irritability than subjects with normal concentrations of A β 42 (agitation: 20% *v.* 12%, OR 1.6, 95% CI 1.1–2.3, $p < 0.014$; irritability: 32% *v.* 18%, OR 2.2, 95% CI 1.5–3.3, $p < 0.001$). None of the CSF markers was related to sleeping or eating problems.

Discussion

This is the first study to investigate the relationship between CSF markers for AD and neuropsychiatric symptoms in subjects with MCI. Abnormal concentrations of t-tau and A β 42 were associated with symptoms of anxiety, whereas none of the CSF parameters was related to symptoms of depression or apathy.

The finding that symptoms of anxiety were more common in subjects with abnormal concentrations of t-tau and A β 42 implies that symptoms of anxiety could at least partially be explained by AD pathology in the brain. This finding may be a direct effect of AD pathology on parts of the brain or may represent neurochemical deficits involved in anxiety, such as neurotransmitter deficits (Lanari *et al.* 2006). Alternatively, anxiety may be a psychological reaction to the insight into their cognitive decline (Schmand *et al.* 1996; Barnes *et al.* 2006), or an anxiety-induced hypothalamic–pituitary–adrenal axis dysregulation that could affect the AD pathology (Sierksma *et al.* 2010).

Post hoc analyses showed that subjects with anxiety did not have a lower baseline MMSE score than subjects without anxiety. In addition, immediate and delayed recall scores of a verbal word-learning test were not different between subjects with and without anxiety, nor were years of education, gender, age and

APOE-e4 carriership. This makes it unlikely that the increase in anxiety with AD CSF markers is a result of more severe impairment. Our findings are consistent with those of several prospective cohort studies that have shown that anxiety is associated with cognitive decline or AD-type dementia at follow-up (Sinoff & Werner, 2003; Palmer *et al.* 2007), but contradict a recent study which found that state anxiety was not a predictor for conversion to AD (Devier *et al.* 2009). Also in our dataset, anxiety was associated with an increased risk for developing AD-type dementia (see below).

We did not find an association between depressive symptoms and CSF markers for AD in MCI subjects. This finding is consistent with a number of previous observations. A neuropathological study showed that depressive symptoms were not related to the load of cortical plaques and tangles and that depressive symptoms did not modify the relationship between amyloid burden and clinical AD (Wilson *et al.* 2003). Furthermore elderly depressed women had increased A β 42 levels in the CSF, whereas AD is characterized by decreased A β 42 levels (Gudmundsson *et al.* 2007). In subjects with AD-type dementia, depression was not associated with AD CSF markers (Engelborghs *et al.* 2006; Skogseth *et al.* 2008). In addition, no associations were found between depressive symptoms and whole brain volume, hippocampal volume or white-matter lesions in subjects with probable AD (Berlow *et al.* 2010). Several clinical studies have found that depression in subjects with MCI was not associated with progression to AD-type dementia (Palmer *et al.* 2010; Ramakers *et al.* 2010). In contrast, other studies have reported that depressive symptoms predicted cognitive decline and AD in subjects with MCI (Modrego & Ferrandez, 2004; Gabryelewicz *et al.* 2007; Teng *et al.* 2007). Thus, although depression may be indicative of an increased risk of cognitive decline in specific settings, it is typically not indicative of AD pathology in subjects with MCI. Depressive symptoms in subjects with MCI may be related to other neurodegenerative processes, such as synaptic or neuronal loss, vascular changes, or neurochemical changes, such as neurotransmitter dysfunctioning (Sierksma *et al.* 2010; Wuwongse *et al.* 2010). Depressive symptoms may also be the result of a primary affective disorder because these disorders are often associated with cognitive impairments. Nevertheless, the presence of these symptoms does not exclude imminent AD (Visser *et al.* 2000).

Symptoms of apathy were also not related to CSF markers for AD. In the DESCRIPA cohort, symptoms of apathy were even more common in subjects with normal A β 42 concentrations. This finding is not consistent with those of a previous study that found a

correlation between t-tau levels and apathy in subjects with mild AD (Skogseth *et al.* 2008), nor is this finding consistent with those of studies that found that subjects with apathy had an increased risk of AD (Robert *et al.* 2006; Feldman *et al.* 2007; Palmer *et al.* 2010). Differences in these findings could be the result of different scales to measure apathy or whether findings were corrected for covariates, such as age and gender. In addition, different mechanisms could underlie symptoms of apathy in subjects with MCI and AD, where in a later stage of the disease, apathy may be the result of degeneration of frontal circuits and white-matter lesions, and more severe cholinergic dysfunctioning (Landes *et al.* 2001; Starkstein *et al.* 2009).

In line with previous studies, agitation, irritability, and problems with sleep or appetite were frequently reported (Apostolova & Cummings, 2007). In the pooled cohort, symptoms of agitation and irritability were associated with abnormal A β 42 concentrations, suggesting that these symptoms may be related to AD pathology. These symptoms, however, were not related to abnormal tau concentrations. The lack of an association of irritability and agitation with tau concentrations, while it correlated with A β 42 concentrations, may indicate that these symptoms occur earlier in the course of AD than anxiety. Alternatively, the lack of an association with tau may be a power issue as the prevalence of abnormal tau was lower than that of abnormal A β 42, probably due to a lower sensitivity of abnormal tau for AD compared with A β 42 (Shaw *et al.* 2009). Problems with sleep or appetite were not related to any of the CSF markers, suggesting that these symptoms are unrelated to AD pathology in the MCI stage.

To further explore the relationship between neuropsychiatric symptoms and AD, we performed a number of *post hoc* analyses. First, we tested the predictive accuracy of neuropsychiatric symptoms for the onset of AD-type dementia at follow-up using Cox regression with correction for age, gender and education in subjects with MCI from the pooled DESCRIPA and ADNI cohorts ($n=565$, also including subjects without CSF). These analyses showed that anxiety (hazard ratio 1.6, 95% CI 1.1–2.1, $p=0.005$) and agitation (hazard ratio 1.9, 95% CI 1.4–2.5, $p<0.001$) were related to an increased risk for developing AD-type dementia, while depression, apathy, and irritability were not. These findings support our observed correlations between neuropsychiatric symptoms and CSF markers, except for irritability. Second, we correlated the NPI symptoms with hippocampal atrophy as assessed with the LEAP (learning embeddings for atlas propagation) score in the pooled cohort with correction for age and gender (Wolz *et al.* 2010; Vos *et al.* 2012). These analyses showed that anxiety, agitation,

irritability and apathy were not associated with hippocampal atrophy. Subjects with depression had significantly larger hippocampal volumes than subjects without depression ($p=0.036$). The lack of an association between anxiety, agitation and irritability with hippocampal atrophy may be explained by the observation that atrophy is a relatively late event in AD compared with CSF biomarkers (Vos *et al.* 2012). The observation that depression was associated with less atrophy supports our observation and that of other studies as discussed above.

We conducted the present study in a pooled sample of two large cohorts. The variability in study design may have introduced a bias. Nevertheless, the association between CSF markers and neuropsychiatric symptoms did not differ between the samples. The fact that the association between anxiety and CSF markers was present in each cohort, despite differences in study design, increased the robustness of this finding. In addition, some variability might be caused by the use of different platforms for measuring CSF A β 42 and tau concentrations in both cohorts. However, recent studies showed that the absolute values of both platforms highly correlate (Reijn *et al.* 2007; Shaw *et al.* 2009; Fagan *et al.* 2011) and identified individuals with underlying pathology equally well (Fagan *et al.* 2011). In the DESCRIPA cohort, the CSF of six subjects was not collected in polypropylene tubes, which might have affected the concentrations of A β 42. We corrected for this in the analyses. In addition, *post hoc* analyses in which these subjects were excluded from the analyses showed similar results. Also, the small differences in inclusion criteria of both cohorts did not affect the results, as *post hoc* analyses in the DESCRIPA cohort selecting subjects with amnesic MCI or selecting subjects with a CDR memory score of 0.5 resulted in similar findings. Medication use, such as the use of acetylcholinesterase inhibitors (AChEIs), N-methyl-D-aspartic acid (NMDA) antagonists, antidepressants or anxiolytics could have affected our results. In the DESCRIPA cohort, none of the subjects used AChEIs or NMDA antagonists. In the ADNI cohort, 86 subjects (32%) used AChEIs or NMDA antagonists. After correction for AChEI or NMDA antagonist use, results remained the same. Antidepressants or anxiolytics were used by 45 subjects in the pooled cohort. After correction for the use of antidepressants or anxiolytics, results remained essentially the same. This indicates that the use of psychoactive drugs did not confound our findings. Our analyses were not corrected for multiple testing. Nevertheless, even a very conservative Bonferroni correction ($p<0.004$) would result in comparable main conclusions about the relationship between the presence of anxiety and abnormal AD CSF markers in the pooled cohort.

One strength of the present study was the large sample size. Neuropsychiatric symptoms were measured with the NPI (Cummings *et al.* 1994), a well-known and commonly used informant-based assessment for measuring neuropsychiatric symptoms. Subjects were included from multi-center studies, increasing the generalizability of the results. However, because these subjects were selected from memory clinics or research settings, the generalizability of these findings to other population-based studies or primary care settings may be limited.

The relationship between CSF AD markers and symptoms of anxiety in subjects with MCI suggests that the course of cognitive functioning in these subjects should be monitored as these subjects could suffer from underlying AD pathology. The high prevalence of neuropsychiatric symptoms (59% in the present study) emphasizes the importance of a psychiatric examination as part of the regular diagnostics for the evaluation of cognitive impairments.

While our study showed a cross-sectional relationship between AD CSF markers and neuropsychiatric symptoms, longitudinal studies would be helpful to investigate the relationship between changes in CSF markers, neuropsychiatric symptoms and the risk of cognitive decline and AD.

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Declaration of Interest

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