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# The Prevalence of Amyloid Pathology in Non-Demented Adults:

## A Meta-analysis and participant-level data analysis


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**ABSTRACT**

Amyloid pathology is thought to be the earliest pathological event in Alzheimer's disease (AD) occurring years before dementia onset. Prevalence estimates of amyloid pathology in subjects without dementia differ widely across studies. The aim of the present study was to estimate the post-mortem prevalence of amyloid pathology in non-demented subjects with a meta-analysis and to identify risk factors for amyloid pathology with a participant-level data analysis.

Medline and Web of Science databases were searched to identify relevant studies. Titles and abstracts were reviewed and all relevant studies were further analysed. Thirteen studies representing 1583 subjects met inclusion criteria. Pooled estimates of the prevalence were obtained using random-effects models and meta-regression was performed.

A total of 1207 subjects in the National Alzheimer's Coordinating Center (NACC) Uniform Data Set and Neuropathology Data Set diagnosed with normal cognition or mild cognitive impairment (MCI) were included in the participant-level analysis. Generalized Estimating Equations (GEE) were used to examine whether amyloid pathology was associated with several risk factors.

The prevalence of amyloid pathology in the total study cohort was 32.1%. Meta-regression showed that increased age at death and enhanced neurofibrillary tangle (NFT) staging in the total study cohort was associated with a higher amyloid prevalence.

In our participant-level analysis we found that MCI subjects had a higher estimated prevalence (49%) compared with cognitively normal subjects (40%). Cognitively healthy and MCI subjects differed significantly in amyloid pathology depending on Apolipoprotein E (ApoE) genotyping. Amyloid pathology differed significantly with age in subjects with or without NFTs and in subjects with or without diffuse plaques.

Assuming that individuals with amyloid pathology are on the path to AD, this study indicates that AD is a major health problem. The identification of predictive factors for the prevalence of amyloid pathology can help to select subjects for future AD prevention studies.

## Introduction

Alzheimer's disease (AD) dementia is the most common neurodegenerative disease and is a fast growing worldwide epidemic (Alzheimer's Association, 2013). It is an irreversible chronic brain disease characterized by progressive decline of several cognitive domains (Hampel, Prvulovic, et al., 2011). AD is a complicated and (genetically) heterogeneous disorder. Individuals with AD dementia often need a high level of care. The increasing amount of elderly in the population has major implications for individuals and creates new medical, political, social and economic challenges to society. Individuals diagnosed with AD dementia need help with basic activities and in the final stages of the disease they will lose their ability to communicate, fail to recognise their loved ones and need continuous healthcare. AD has an increasing impact on society nowadays, therefore the focus of research has changed toward younger individuals and the earlier phases of cognitive deterioration and mild cognitive impairment (MCI) (Rocca et al., 2011). Today, the aim is to detect pathological mechanisms, which are fundamental in dementia, and to slow the conversion of cognitive deterioration and MCI to full dementia.

### *Prevalence and incidence*

Hebert, Weuve, Scherr and Evans (2013) estimated that the prevalence of AD dementia for people aged 65 years and older was 4.7 million in America in 2010. Of these, 15% was between 65 and 74 years, 48% was between 75 and 84 years and 38% was 85 years or older (Hebert, Weuve, Scherr, & Evans, 2013). The growing number of AD cases is attributable to the continued increase in size of the older population. Within a few decades a large quantity of people will enter the age group at which the incidence of AD dementia is highest. Additionally, Hebert et al., (2013) estimated that 13.8 million people over the age of 65 will suffer from AD dementia in 2050, with 50.7% of these aged 85 years or older.

*Risk factors*

There are several factors that elevate the risk of developing AD. To date, it is widely recognized that an important risk factor for AD development is age. Family history is also a risk factor for the development of AD (Alzheimer's Association, 2013). Individuals with a first-degree relative with AD are at increased risk. Another risk factor implicated in the pathogenesis of AD is the ε4 allele of apolipoprotein E (ApoE) (Ghebremedhin et al., 2001). This genetic risk factor is associated with late-onset AD. Carriers of at least one ApoE ε4 allele are likely to develop late-onset AD at an earlier age compared to non-carriers (Myers et al., 1996; Slooter et al., 1998). However, presence of the ε4 allele is neither necessary nor sufficient for developing AD (Myers et al., 1996). Furthermore, there are important behavioural risk factors for the development of AD, such as cardiovascular health, stress and Diabetes Mellitus (DM) (Arvantakis, Wilson, Bienias Evans & Bennett, 2004; Peila, Rodriguez & Launer, 2002; Rodrigue et al., 20013). Hypertension enlarges the risk for development and progression of AD (Kivipelto et al., 2001). A growing number of studies indicate that an expanded period and/or a higher stage of hypertension is associated with an increased risk for AD in later life (Feldstein, 2012). Chronic stress can accelerate the emergence of AD (Machado et al., 2014). Stress hastens cognitive impairments and augments the deposition of extracellular amyloid, and is therefore a behavioural risk factor for the development of AD (Cutler et al., 2003).

*Stages of Alzheimer's disease*

The clinical disease stages of AD have been divided into three phases (Jack et al., 2010). First, there is an asymptomatic or preclinical stage. In this phase, there are measurable changes in the brain and cerebrospinal fluid (biomarkers). These changes indicate the earliest signs of the disease, but there is no evidence of cognitive symptoms (Dubois et al., 2010). The next stage is the prodromal stage, commonly known as mild

cognitive impairment (MCI) (Jack et al., 2010). It is featured by the onset of the first cognitive impairments. There are mild, but measurable changes in thinking abilities and episodic memory that are notable to the person affected and the people in their surroundings, however not sufficiently severe to affect instrumental activities of daily living (Dubois et al., 2010). There is also evidence of the presence of AD pathological changes at this stage, such as a slightly increased number of amyloid plaques and occasionally a higher staging level of neurofibrillary tangles. The dementia stage is the most severe stage of AD. It is characterised by prominent amnesia with additional impairments in language and semantic knowledge, executive function, attention, and constructional and visuospatial abilities (Bondi et al., 2008). The impairments in several cognitive domains are severe enough to produce loss of function and frequently result in behavioural changes. Therefore, these interfere with social function and/or activities of daily living. "It is ultimately leading to a loss of independence and causes a heavy personal toll on the patient and the family" (Bateman et al., 2012). In the 'end-stage' of AD only basic biologic functions are preserved.

*Neuropathology of Alzheimer's disease*

AD is neuropathologically characterized by Aβ peptide deposited extracellularly in amyloid and senile plaques (SPs), also called neuritic and diffuse plaques, and by intracellular neurofibrillary tangles (NFTs), composed of accumulation of hyperphosphorylated tau (p-tau) protein (Mungas, Tractenberg, Schneider, Crane, & Bennett, 2014; Oddo et al., 2006). These lesions are commonly found during post-mortem examination in demented patients with AD. Further, "they are associated with neuronal loss, synaptic loss, and frequently with cerebral amyloid angiopathy" (Thal et al., 2013). As indicated above, there is evidence suggesting that AD begins with a long asymptomatic period during which the pathological process is proceeding (McKee et al., 2006). The pathologic lesions of AD might develop up to 20 years before the clinical discovery of

cognitive impairment (Knopman et al., 2003). Occasionally, this pathological process becomes symptomatic during life. Amyloid pathology is thought to be the earliest pathological event in AD and may lead to reduced cell function, the formation of NFTs, cell death, cognitive impairment and clinical dementia (Jack et al., 2010). Neuropathological measures for AD depend on the concentration of SPs and NFTs to differentiate between AD and normal aging. Thal et al. (2013) demonstrated that amyloid and senile plaques are generally accompanied with NFTs and are indicative for asymptomatic AD. The neuropathological lesions agglomerate many years before sufficient synaptic and neuronal damage occurs to produce the symptoms of AD (Price et al., 2009). Therefore, these non-demented individuals are seen as at risk for developing AD.

*The prevalence of amyloid pathology*

Amyloid pathology in cognitively normal individuals first captured the attention of researchers in the 1990s. Since then, a large number of studies concerning the amyloid burden in healthy older adults emerged. This growing interest has led to the development of preclinical AD biomarkers. These biomarkers are used to determine if individuals are at increased risk for conversion to dementia (Caselli & Reiman, 2013). Brain imaging and cerebrospinal fluid (CSF) are the most used biomarkers to promote early detection, prediction, tracking and scientific study of AD (Hampel, Wilcock, et al., 2011). CSF surrounds the brain and is rich in waste products of the brain. It could serve as a mirror for what kind of damage processes occur in the brain. At the end of last century they started measuring Aβ-42 and tau in CSF. These studies discovered reductions in CSF Aβ-42 levels in the preclinical stages of AD. The decrease in Aβ-42 levels reflects amassment of the Aβ peptide in neuritic plaques (Caselli & Reiman, 2013). Another well-established measurement for the preclinical detection of AD is amyloid-PET (positron emission tomography). This imaging technology is used to directly measure the amount of amyloid

plaques in the brain of living individuals (Reiman & Jagust, 2012). Amyloid-PET involves the injection of a chemical into the bloodstream that is radioactive for a very short time and seeks out and binds to amyloid deposits. Using biomarkers for the measurement of Aβ levels in the brain will lead to a greater certainty of the right diagnosis and will result in detection in an earlier stage of the disease. Preclinical biomarkers promise to help define the preclinical stages of AD (Caselli & Reiman, 2013). In addition, biomarker studies resulted in a possibility to understand the destruction processes that are going on during life before an individual develops clinical AD or before death. It can extend our base information about the underlying pathogenesis of the disease. They have the potential to further characterize the route of different biological changes linked with the predisposition to AD (Caselli & Reiman, 2013).

A definitive diagnosis of AD can only be obtained after post-mortem examination. The diagnosis of AD at autopsy relies on the presence of amyloid plaques and neurofibrillary tangles in the brain, as explained above. Cognitively normal individuals who have histological lesions of AD found at autopsy, are considered to have had preclinical AD (McKee et al., 2006). Post-mortem assessment to determine the amyloid burden in brains of cognitively healthy individuals is crucial for a better comprehension of the diagnostic concepts and the development of preventive therapeutic interventions. The prevalence of non-demented individuals with amyloid pathology has been estimated around 20-40% (Aizenstein et al., 2008; Jack et al., 2010), but differs widely across studies. The estimated amyloid prevalence in MCI subjects lies between 3-50% (Graham et al., 1997; Lopez et al., 2003). This variation may be attributed to the following reasons. First, the neuropathological criteria to determine whether an individual has increased amyloid levels vary between studies. Second, the population involved is different in each survey. Moreover, different

samples of gender, age at death, degree of education, inclusion or exclusion criteria may have led to divergent prevalence estimates.

To date, there are four published criteria for the postmortem neuropathological diagnosis of AD; Khachaturian criteria (Khachaturian, 1985), Washington University criteria (McKeel et al., 2004), The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (Mirra et al., 1991), and the National Institute of Aging and the Reagan Institute of the Alzheimer's Association criteria (The National Institute on Aging, 1997). NFTs are typically staged according to the classification of Braak and Braak (1991). Most studies use one set of criteria or a combination of these criteria to estimate the neuropathological diagnosis of AD. The criteria all use different cut-off scores to determine whether an individual has no, possible, probable or definite AD. Clearly the observed variance in prevalence rates across studies and the noted differences in group composition, cut-off criteria and inclusion criteria call for a further systematic, more inclusive study of amyloid pathology in the non-demented population. To this aim we conducted a meta-analysis. The present study estimated the post-mortem prevalence of amyloid pathology in cognitively healthy individuals and MCI by age category and ApoE ε4 status with a meta-analysis. Furthermore, the effect of risk factors on amyloid prevalence was tested using participant-level data. The data used for the meta-analysis did not provide subject-level data. Therefore, we obtained data for the participant-level analysis from the National Alzheimer's Coordinating Center (NACC) Uniform Data Set (UDS) and Neuropathology Data Set. These data of non-demented individuals and MCI subjects were collected at 36 existing and former Alzheimer's Disease Centers. These data were used to determine the predictive factors of amyloid prevalence in healthy control individuals and in MCI subjects.

## Methods

### *Meta-analysis*

#### *Literature search*

Literature search was performed in the MEDLINE and Web of Science databases. We searched for articles published up to January 2014 to include as much articles as possible. The search terms were 'neuropath*' and 'plaques' or 'amyloid' and 'Alzheimer*'. Additionally, we excluded animal topics, like 'rat', 'mouse', 'mice', 'monke*', 'cat', 'dog', 'animal' and 'canin*'. Other eligible articles were identified by searching the references cited in the acquired published articles. We did not specifically search for unpublished articles or abstracts. Only studies published in English were considered for analysis. We used the following screening procedure: a) titles were reviewed to determine potential articles related to our topic; b) abstracts were reviewed to diminish the number of articles; c) full text of the articles was read to distinguish related articles; d) the reference list was checked to identify related articles. Articles meeting inclusion criteria were then independently reviewed, rated and organized according to study cohort.

#### *Study selection*

Studies were included if they met the following criteria: a) the study sample consisted at least for a part of non-demented individuals, control subjects or MCI subjects; b) the number of participants in de study is more than 10; c) the study includes data on prevalence or there is an opportunity to contact the authors to provide these data; d) there was an a priori defined cut-off point for amyloid abnormality; e) participants in the study sample were free of clinical dementia at the last evaluation before death; e) all participants received an assessment of cognitive functioning at baseline. In contrast, studies were excluded when the cohorts or subjects were overlapping or when the studies concerned other neurological or psychiatric diseases.

*Data extraction*

We completed a data-extraction form for each study. For more information regarding the data-extraction form, see appendix.

*Diagnosis*

Normal cognition was defined as the absence of cognitive impairment at age at death. This means a normal neuropsychological test performance at baseline and at their last clinical evaluation before death. Neuropsychological test scores had to be above standard clinical cut points for dementia. Subjects with normal cognition had no impairment in social or occupational functioning and there was no decline in performance. MCI was defined according to existing criteria of Petersen (2004). MCI subjects had some cognitive impairment but of insufficient severity to constitute dementia (Petersen, 2004). There are mild changes in cognitive functioning that are notable to the individual himself and the people in their surroundings, however not sufficiently severe to affect their activities of daily living (Dubois et al., 2010). When cognitive status was defined as non-demented or not explicitly specified as normal cognition or MCI, we defined these subjects as 'non-demented'.

*Setting*

We classified the studies according to setting in clinical studies, population-based studies, community studies, research studies and other studies.

*Amyloid Assessment*

For assessment of amyloid pathology we maintained the criteria of The Consortium to Establish a Registry for Alzheimer's Disease (CERAD), because it is much more commonly used than the other existing criteria for the detection of amyloid pathology. "CERAD is based on the specific assessment of only neuritic plaques, a subset of Aβ deposits that contain dystrophic neurites which are thought to reflect more mature plaque pathology"

(Boluda et al., 2014). CERAD scores are based on a semiquantification of neuritic senile plaques density. The CERAD criteria are adjusted for age and indicate three levels of certainty that dementia is explained by the neuropathological diagnosis or that dementia is absent: no, possible, probable and definite AD (Price et al., 2009). The diagnosis of no or possible AD were considered as normal amyloid pathology in our study, whereas the diagnosis of probable or definite AD were considered as abnormal amyloid pathology.

NFTs were staged according to the classification of Braak and Braak (1991). Briefly, Braak stage 0 accord with no NFTs, stages I-II indicate NFTs in the transentorhinal and entorhinal regions, stages III-IV corresponds with NFTs in the hippocampus and inferior temporal cortex and stages V-VI indicate NFTs distributed in the isocortex (Berr et al., 2001; Price et al., 2009). The first two NFT stages have never been associated with the clinical symptoms distinctive of AD. Therefore, the first two stages of Braak and Braak (1991) indicate an absence of NFTs in our study. Stages III-VI are intermediate to severe NFT stages and were considered as abnormal, indicating the presence of NFTs.

*Characteristics*

Subjects were ApoE positive when they have at least one ε4 allele. ApoE genotyping was available for sixty-four subjects in the total study cohort. Years of education was available for 190 subjects.

*Data Analysis*

Data were imported into the STATA (version 12) meta-analysis program and a pooled estimate of amyloid prevalence was calculated and a forest plot was obtained. To determine whether to use the fixed-or random effects model, statistical heterogeneity between and within groups was measured using Cochran's Q statistic. The Q test was significant, which means we assumed a random-effects model with inverse variance weighting. We calculated pooled prevalence estimates and confidence intervals. Meta-regression analysis for the total

study cohort was performed and stratified meta-regression separate for the three different diagnostic groups was performed to analyse the relationship between amyloid prevalence and year of study, age at death, setting gender, years of education and ApoE genotype.

*Data from the National Alzheimer Coordinating Center*

*Study subjects*

The study population was compiled of participants from the National Alzheimer's Coordinating Center (NACC) Uniform Data Set (UDS) and Neuropathology Data Set, gathered from approximately 36 existing and former Alzheimer's Disease Centers (ADCs) in the United States (Beekly et al. 2007). The NACC database includes information on participants with normal cognition, mild cognitive impairment (MCI), Alzheimer's Disease (AD) dementia and other dementias. Subject demographics and standard motor, behavioral, functional, and neuropsychological assessments have been included in the UDS (Serrano-Pozo et al., 2013). Subjects underwent cognitive and functional testing. Standardized forms were used and all subjects and informants gave informed consent. The Neuropathology Data Set is a specific data area of the extensive UDS. For the subjects included in the UDS who had autopsy, neuropathological characteristics were adopted and submitted to the NACC.

Subjects were included when they met the following inclusion criteria: a) no clinical dementia at age of death; 2) underwent autopsy; 3) had an assessment of cognitive functioning at baseline to determine if subjects had normal cognition or MCI. Exclusion criteria were in vivo diagnosis of dementia (e.g. dementia with Lewy bodies, vascular dementia, Parkinson disease, ischemia, etc.) or cardiovascular disease, seizures, traumatic brain injury, history of stroke or other psychiatric diseases (e.g. alcohol use, depression, etc.) (Beekly et al. 2007).

*Data Collection*

Several factors were regarded as potential sources affecting the presence of neuritic plaques in subjects. Demographic characteristics used in this study included gender, years of education and age at death. Clinical characteristics included clinical diagnosis at death and ApoE genotyping. The neuropathological features assessed included Braak & Braak NFT staging and diffuse plaques. For statistical analysis, the variable 'age at death' was treated as a continuous variable. We dichotomized 'education' at the mean (15 years) of our total sample (high >= 16 years versus low < 16 years). The categorical variable 'diagnosis' was, as indicated above, divided in two separate groups; cognitively healthy and MCI. The categorical variable 'ApoE' was divided into those who did and those who did not carry at least 1 ε4 allele. 'Neuritic plaques' was chosen as the outcome variable because it measures the presence or absence of neuritic plaques in different brain areas measured with CERAD criteria. To check for influence of Braak & Braak NFT staging on neuritic plaques, the categorical variable 'NFT' was divided into two groups: those without NFTs or those with stage I & II and those with stage III or higher. 'Diffuse plaques' was split into present (moderate or frequent diffuse plaques) or absent (sparse or no diffuse plaques).

*Statistical analysis*

Generalized linear models of neuritic plaques with a logit link function were fit to the data from the NACC database. The relationship between each variable and neuritic plaques status (presence versus absence) was evaluated using generalized estimating equations (GEE). GEE was used to account for clustering of subjects in Alzheimer's Disease Centers (ADCs). The regression models were run with an exchangeable correlation structure and the regression parameter used is the Wald test with robust standard errors. Results were considered significant at a 0.05α level. All two-way and three-way interactions were tested.

Age was an important risk factor, therefore it was used as a covariate in all models. In case of significance, risk factors were used as a factor in the subsequent models.

## Results

### *Meta-Analysis*

The search yielded abstracts of 2339 publications. After reading the titles and abstracts, 2180 articles were excluded for various reasons (such as reporting on other illnesses, published before 1985 or reviews), leaving 159 articles for revision. Thirteen of the 159 articles had no online full text version accessible. We found ninety-two overlapping cohort studies. Eventually, forty-three publications with no overlapping cohort satisfied all inclusion criteria. There were many publications with incomplete data, leaving thirteen for further analysis. This included four publications with data of only subjects with normal cognition, two publications with data of only MCI subjects, three with data of only non-demented individuals, one with data of subjects with normal cognition or non-demented subjects and three with data of both non-demented and MCI subjects.

The thirteen included studies represented 1583 subjects, of whom 391 were subjects with normal cognition, 1040 non-demented individuals and 152 MCI patients. The pooled mean baseline characteristics of the total study cohort were as follows: age 81,5 years (range 71-89), 55.9% female (*n*= 885) and education 14.8 years (range 9-19). The baseline characteristics of each diagnostic group per study are shown in table 1. The pooled mean characteristics per diagnostic group are shown in table 2.

**Table 1**

*Characteristics for each group of subjects per study included in the meta-analysis*

| Author | Publication Year | Study | Mean age [1] (years) | % Female | Mean education (years) | Criteria | Abnormal | Normal | % NFT abnormal |
|---|---|---|---|---|---|---|---|---|---|
| *Control subjects* | | | | | | | | | |
| Knopman | 2003 | MAYO | 85.4 | 61.5 | 13.1 | CERAD | 7 | 32 | 12.8 |
| Noda | 2006 | Hisayama | 84.5 | 54.5 | - | CERAD & Braak | 5 | 39 | 25.0 |
| Peisah | 2007 | Sydney/ NSW | 75.5 | - | - | CERAD | 36 | 152 | 13.8 |
| Purohit | 2011 | Mumbai | 71.1 | 45.1 | - | CERAD | 9 | 82 | - |
| Tiraboschi | 2004 | California | 80.2 | 41.4 | 15.9 | CERAD | 11 | 18 | - |
| *Non-demented subjects* | | | | | | | | | |
| Abner | 2011 | Nun Study | - | 100.0 | - | CERAD & Braak | 35 | 221 | 13.7 |
| Bennett | 2005 | Religious Orders Study | 81.8 | 50.0 | 18.7 | CERAD | 28 | 32 | 55.0 |
| Brayne | 2009 | CC75C | 87.7 | 61.0 | - | CERAD & Braak | 76 | 7 | - |
| McKee | 2006 | FHS | 81.5 | 48.0 | - | NIA-Reagan | 4 | 21 | 16.0 |
| Murayama | 2004 | Tokyo | 80.5 | 38.6 | - | CERAD | 21 | 168 | 0.5 |
| Purohit | 2011 | New York | 71.0 | 30.8 | - | CERAD | 2 | 89 | - |
| Sonnen | 2013 | - | 87.0 | 60.1 | - | CERAD | 158 | 178 | 37.5 |
| *MCI subjects* | | | | | | | | | |
| Bennett | 2005 | Religious Orders Study | 85.0 | 59.5 | 18.5 | CERAD | 23 | 14 | 83.8 |
| McKee | 2006 | FHS | 86.3 | 100 | - | NIA-Reagan | 3 | 0 | 100.0 |
| Murayama | 2004 | Tokyo | 80.1 | 47.1 | - | CERAD | 23 | 64 | 8.0 |
| Petersen | 2006 | MAYO | 88.9 | 66.7 | 13.5 | CERAD | 3 | 12 | 60.0 |
| Stephan | 2012 | MRC CFAS | 78.3 | 30.0 | 9.2 | CERAD | 5 | 5 | - |

[1] Age at death

- Indicates missing data

**Table 2**

*Pooled mean characteristics per diagnostic group in the meta-analysis*

| | **Normal Cognition** | **Non-demented** | **MCI** |
|---|---|---|---|
| Age (years) [1], (range) | 79.3 (71-86) | 81.6 (71-88) | 83.7 (78-89) |
| Gender, n female, (% female) | 198 (50.6%) | 577 (55.5%) | 92 (60.7%) |
| Education (years), (range) | 18.7 (18-19) | 18.7 (18-19) | 13.7 (9-19) |

[1] Age at death

The aggregated prevalence estimate of the total study cohort was 32.1% (95% CI 19.3% to 45.0%) (Figure 1). Prevalence estimates were 16.9% (95% CI 10.1 to 23.7) for the subjects with normal cognition, 32.6% (95% CI 9.5 to 55.7) for the non-demented subjects, and 45.9% (95% CI 24.5 to 67.3) for MCI subjects. Meta-regression did not show a significant association between prevalence and diagnostic group ($p>0.05$). Though, meta-regression showed some other associations. Increasing age at death in the total study cohort was associated with a higher prevalence estimation ($p<0.05$). Likewise, percentage abnormal NFT staging significantly increased the prevalence estimation ($p<0.05$) (Figure 2). In the three subject groups separately, there were no significant associations.

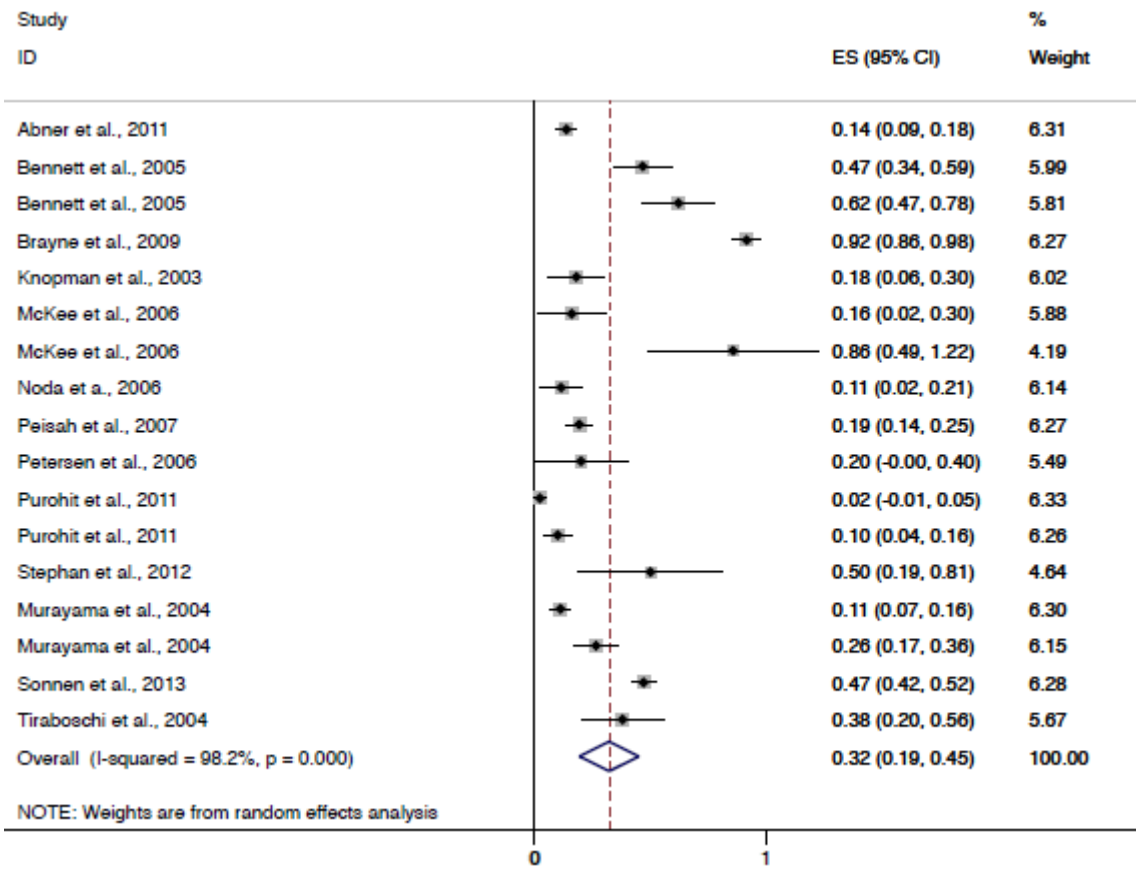
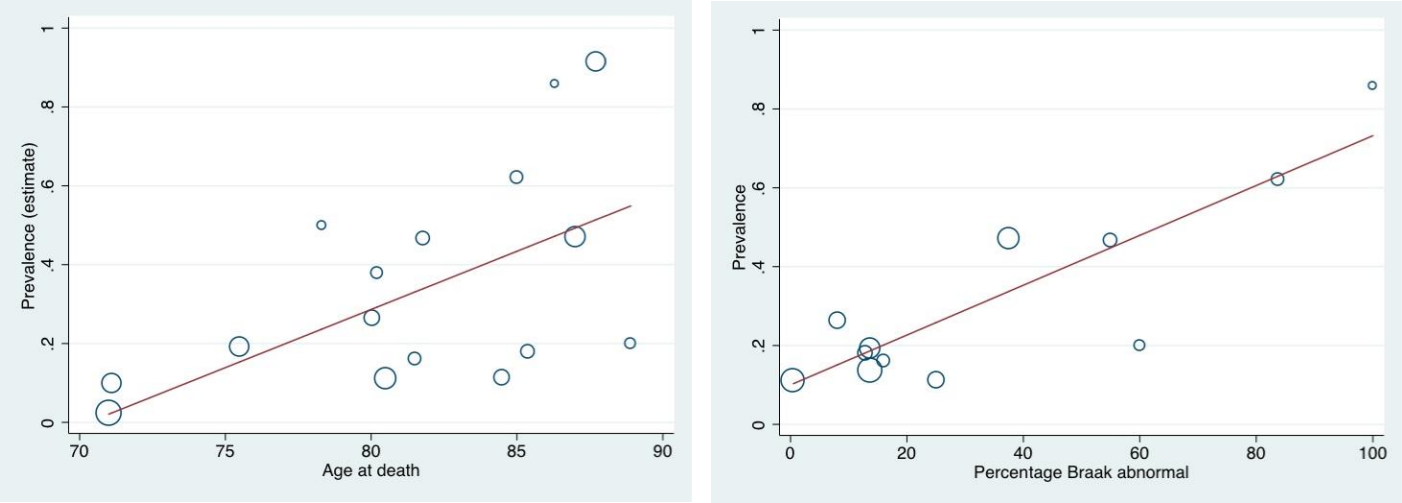



**Figure 1.** Forest Plot of baseline prevalence of amyloid pathology in the total study cohort. The squares represent the weight of the particular study in the overall analysis. The horizontal line across the squares indicates the 95% Confidence Intervals (CI). The diamonds represent the overall effects. Studies occurring twice in the figure yielded data on MCI subjects and on individuals with normal cognition. In each case, the first reference represents the subjects with normal cognition and the second reference represents the MCI subjects.




a. b.

**Figure 2.** Meta-regression analysis indicated that age at death and percentage NFT staging abnormal were statistically significant related to the prevalence of amyloid pathology in the total study cohort. a) Relationship between age at death and amyloid prevalence. b) Relationship between percentage NFT staging abnormal and amyloid prevalence.

*NACC database*

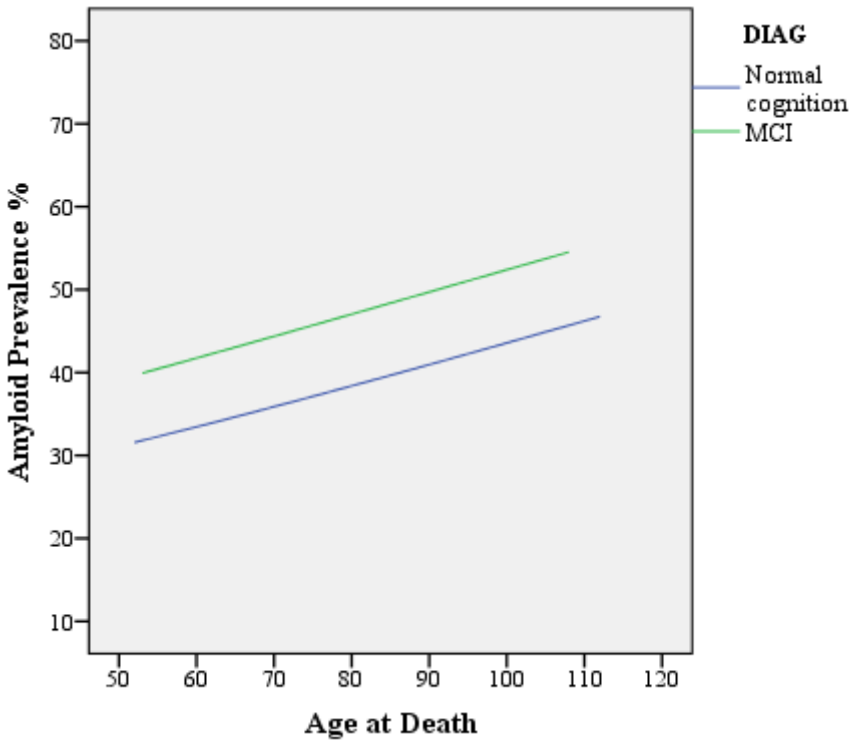
A total sample of 1207 participants enrolled since 2005 met inclusion criteria for our study and were therefore used to study the relationship between the covariates and our outcome variable using the participant-level data from the NACC. Among the 1207 participants, 826 (68.4%) were subjects with normal cognition and 381 (31.6%) were subjects diagnosed with MCI. Subjects demographic and clinical characteristics are given in Table 3.

**Table 3**

*Participant demographic and clinical characteristics from the NACC database.*

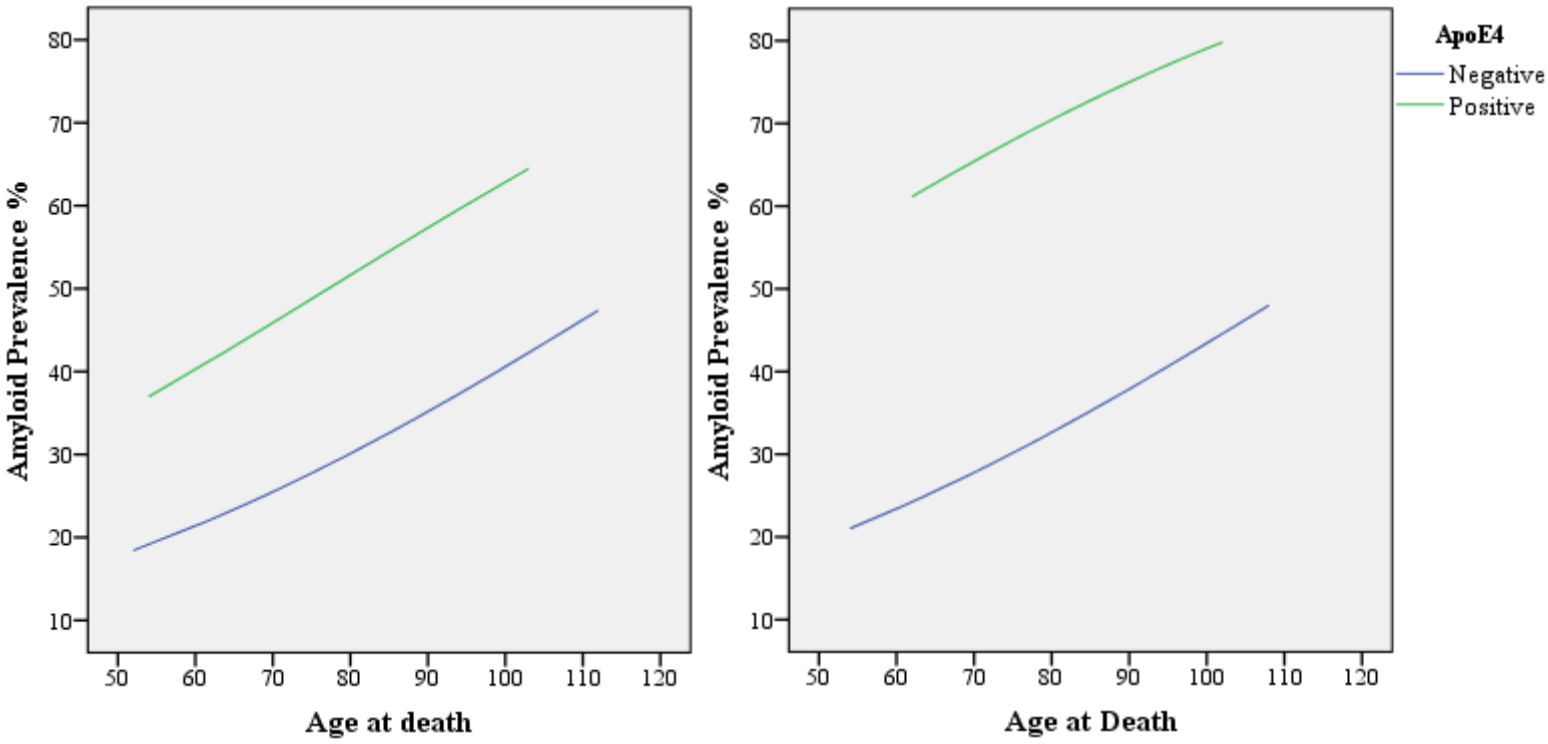
| | Normal cognition (*n* =826) | Missing *n* (%) | MCI (*n* =381) | Missing *n* (%) | Total (*n* =1207) | Missing *n* (%) |
|---|---|---|---|---|---|---|
| Age at death | 87.3 (8.0) | 0 | 86.9 (9.4) | 0 | 87.2 (8.5) | 0 |
| Sex: female, *n*(%) | 494 (59.8%) | 0 | 189 (49.6%) | 0 | 683 (56.6%) | 0 |
| Education (years) | 16.6 (7.7) | 0 | 16.4 (7.0) | 0 | 16.5 (7.5) | 0 |
| ApoE: ε4 allele present, *n*(%) | 156 (18.9%) | 116 (13.9%) | 82 (21.5%) | 86 (22.6%) | 238 (19.7%) | 201 (16.7%) |
| Neuritic plaques present, *n*(%) | 353 (42.7%) | 2 (0.2%) | 204 (53.5%) | | 557 (46.1%) | 2 (0.2%) |
| Diffuse plaques present, *n*(%) | 343(41.7%) | 122 (14.8%) | 172 (45.1%) | 62 (16.3%) | 515 (42.7%) | 184 (15.2%) |
| NFT Braak present, *n*(%) | 456 (55.2%) | 4 (0.5%) | 270 (70.9%) | 2 (0.5%) | 726 (60.1%) | 11 (0.8%) |

First we tested if age at death and diagnosis had an effect on amyloid prevalence. Diagnostic group had a significant effect on the prevalence of amyloid pathology ($p$=0.000). Participants with normal cognition had a prevalence rate of 40% and participants diagnosed with MCI had a prevalence rate of 49% at the mean age of 87.2 (graph 1 represents prevalence estimates at all ages). Age at death had no significant effect the prevalence of amyloid pathology.




**Graph 1.** Prevalence estimates of amyloid pathology by age at death and diagnosis. Amyloid pathology was present when the density of neuritic plaques in different brain areas was defined as 'probable AD' or 'possible AD' measured with CERAD criteria.

Second, we tested whether subjects with at least 1 ApoE ε4 (ApoE4) allele differed in prevalence from subjects with no ε4 allele in prevalence, and we examined interactions of ApoE4 with age at death and diagnosis. The diagnostic groups differed significantly in prevalence depending on ApoE4 genotyping ($p$= 0.006), while the effect of ApoE was equal at all ages. In subjects with normal cognition, the estimated prevalence rate for subjects without the ApoE4 genotype was 34%, while ApoE4 carrying subjects had a prevalence of 56%. MCI subjects who were ApoE4 negative had an estimated prevalence of 37%, while subjects who were ApoE4 positive scored 74% (graph 2).




a. b.

**Graph 2.** Prevalence estimates of amyloid pathology by age at death and ApoE4 separately for diagnostic group. a) Cognitively healthy individuals; b) MCI subjects. Amyloid pathology was present when the density of neuritic plaques in different brain areas was defined as ‘probable AD’ or ‘possible AD’ measured with CERAD criteria.

Thirdly, we tested a model with years of education. We tested if highly educated subject differed in amyloid prevalence compared to subjects who were less educated, controlled for diagnosis, age at death and ApoE4. Subjects did not differ significantly in years of education, independent of diagnosis ($p$= 0.196). The estimated amyloid prevalence in subjects with a high education level was 52%, while subjects with a lower education level had a prevalence of 47%.

Fourth, we tested whether subjects with NFTs differed from subjects without NFTs in amyloid prevalence controlled for diagnosis, age at death and ApoE4. Subjects with NFTs had an estimated prevalence of 64% and subjects without NFTs had a prevalence of 34%. Amyloid prevalence differed with age in subjects with or without NFTs ($p$=0.005), independently of diagnosis. The discrepancy in amyloid prevalence in subjects with or without NFTs decreased as age increased.

Finally, we included a model with diffuse plaques. We tested if subjects with diffuse plaques differed from subjects without diffuse plaques in estimated prevalence controlled for diagnosis, age at death and ApoE4. Subjects with diffuse plaques had an amyloid prevalence of 75%, while subjects without diffuse plaques had a prevalence of 14%. The estimated prevalence differed with age in subject with or without diffuse plaques ($p$=0.003) in each diagnostic group. There was also a trend regarding diffuse plaques and ApoE4 genotyping ($p$=0.050), independently of diagnosis. ApoE4 carriers with diffuse plaques tended to have a higher amyloid prevalence than non-carriers with diffuse plaques.

## Discussion

The aim of the current study was to estimate the post-mortem prevalence of amyloid pathology in cognitively healthy individuals and MCI subjects with a meta-analysis and to identify risk factors for amyloid pathology with a participant-level data analysis. We found an estimated amyloid prevalence of 32% in the total study cohort. The prevalence estimates for the cognitively normal subjects was 17%, for the non-demented group with suspected AD it was 33% and the MCI subjects had an estimated amyloid prevalence of 46%. Meta-regression showed that increased age at death and enhanced NFT staging were associated with a higher prevalence estimate in the total study cohort. The participant-level analysis yielded several risk factors for amyloid pathology. Together, we demonstrated that diagnostic group, ApoE4 genotyping, NFT staging and diffuse plaques were predictive factors for the development of amyloid pathology.

### *Meta-analysis*

The results of the meta-analysis showed an estimated post-mortem prevalence of 32.1% in our total study cohort. The prevalence estimates for the three groups separately were 16.9% in the subjects with normal cognition, 32.6% in the non-demented subjects and 45.9% in the MCI subjects. Prevalence estimates of amyloid pathology differed widely across studies (Aizenstein et al., 2008; Jack et al., 2010; Price & Morris, 1999), presumably due to differences in analytical methodology and classification criteria. We tried to overcome this issue by conducting a meta-analysis after creating a common set of variables. Previous studies used one set of neuropathological criteria or a combination of these criteria to estimate the neuropathological diagnosis of AD. Therefore, we classified the amyloid pathology in all our participants in the meta-analysis according to the CERAD criteria. We established one cut-off score to determine whether someone had amyloid pathology or not. Furthermore, differences in prevalence estimates in earlier studies were probably due to

differences of classification criteria for non-demented subjects and MCI subjects. Therefore, we established clear classification criteria to divide our subjects in the right corresponding diagnostic group. The difference in prevalence estimates across studies is probably also due to several AD risk factors. The predictive factors we found for amyloid pathology are based on participant-level data and are explained in more detail below.

Meta-regression showed two associations with increased amyloid prevalence in the total study cohort, namely age at death and NFT staging. Increasing age at death was associated with a higher estimated prevalence. It is widely known that age is an important risk factor for the development of AD (Alzheimer's association, 2013; Bondi et al., 2008; Sloane et al., 2002). The incidence and prevalence of AD increases at an exponential rate at a certain age category, most clearly between ages 60 and 90 (Jorm & Jolley, 1998). Our obtained association between amyloid pathology and age confirms the assumption that amyloid pathology is a precursor of AD. Furthermore, enhanced NFT staging significantly increased the estimated amyloid prevalence. This finding is consistent with previous reports as NFT is considered as one of the main pathological markers of AD. NFT change is even thought to accelerate in the presence of amyloid pathology (Price & Morris, 1999).

Considering the controversy about sex differences in the prevalence of Alzheimer's disease, our meta-regression did not detect a difference in prevalence rate related to gender. Some studies provide evidence that women have a slightly greater risk for the development of AD than man (Bachman et al., 1992; Gao et al., 1998; Launer et al., 1999; Zhang et al., 1990). Other studies showed no differences or found a higher prevalence of AD in men (Fratiglioni et al., 1991). Meta-analysis regarding the prevalence of AD in demented subjects also found no gender differences in prevalence rate (Jorm & Jolley, 1998; Raber, Huang & Ashford, 2004). One study investigating gender differences on amyloid proteins in

cognitively normal individuals found in accord with our findings no effects of gender on amyloid proteins (Kunicki et al., 1998).

Meta-regression showed no associations for the three diagnostic groups separately. In each diagnostic group were only five to seven studies included, which led to a low statistical power in the meta-regression. In accordance, the separate group analysis suffered more than the total group from the relatively large amount of missing data on several variables, like education and even NFT staging. Furthermore, it is possible that the association of age at death on amyloid pathology found in the total study cohort disappeared when we divided the three groups by the fact that the mean age at death was highest in the MCI group, then the non-demented group and was lowest in the cognitively healthy group.

Nevertheless, we assumed there were more predictive factors for the prevalence of amyloid pathology than we could demonstrate with the data of our meta-analysis and meta-regression. Therefore, we compared results with participant-level data from the NACC Uniform Data Set and Neuropathology Data Set. Despite the fact that the subjects in our two analysis are not the same, the results of the analysis are comparable. Both analyses are based on data from multiple centers and the subjects in the meta-analysis did not overlap the subjects in the NACC database.

*Participant-level data*

Our participant-level analysis revealed a difference in the presence of amyloid pathology in the cognitively healthy group compared with the MCI group. Individuals with MCI did show an elevated amyloid prevalence in comparison with cognitively normal subjects. The estimated prevalence for the cognitively healthy group was 40% compared with 49% in the MCI group. These estimated prevalence rates were consistent with earlier post-mortem studies who found a higher estimated prevalence of amyloid plaques in non-demented/cognitively normal subjects compared with MCI subjects (Aizenstein et al., 2008;

Graham et al., 1997; Jack et al., 2010; Lopez et al., 2003). MCI is considered to represent a borderland between normal aging and AD (Bondi et al., 2008; Petersen, 2004). Therefore, it is suitable that MCI subjects have a higher amyloid prevalence as they are considered at an advanced path for the development of AD. In our meta-analysis we did not find an association between diagnostic group and amyloid prevalence, which is in contrast with the results of the participant-level analysis. We only found the tendency that amyloid prevalence was highest in the MCI group, then the non-demented group and was lowest in the cognitively healthy group, but it was not significant. It is possible that the non-demented group in our meta-analysis was too broadly defined. This may have led to an excessive classification of subjects in the non-demented group, whereas some subjects should be classified in either the MCI group or the cognitively healthy group. It is reasonable that due to ambiguous MCI definitions in some studies, we defined these subjects as non-demented in our sample, ultimately leading to a higher amyloid prevalence in this diagnostic group. On the other hand, the prevalence rate of the non-demented group did not reach the level of the MCI group possibly due to misclassification of control subjects in the non-demented group. This is probably the reason that diagnostic group had no association with the estimated amyloid prevalence in our meta-regression.

Furthermore, cognitively healthy subjects and MCI subjects differed in amyloid prevalence depending on ApoE4 genotyping in our participant-level analysis. ApoE4 carriers had a higher prevalence estimation compared to non-carriers. These findings are in accordance with previous studies. ApoE is considered as a major risk factor for the development of AD and since it was first shown to have a major impact on the age at which individuals develop AD, it has been well studied. However, the association of ApoE on AD pathology is different in various ethnic groups (Farrer et al., 1997; Hendrie et al, 2001). Farrer et al. (1997) found a weaker association among African Americans and Hispanics

compared with Japanese subjects. In addition, Hendrie et al. (2001) found only a trend between ApoE and AD in African American subjects. Hence, it is not entirely clear in which way ApoE genotyping is engaged in the aging process. Nevertheless, our association of ApoE genotyping with amyloid pathology suggests that ApoE is an important predictive factor in estimating the prevalence of amyloid pathology in cognitively healthy and MCI subjects, as it is in AD. Notably our meta-regression did not reveal any associations between amyloid prevalence and ApoE4 genotyping, probably due to the great amount of missing data on the ApoE gene in our meta-analysis.

Our study further demonstrates that NFTs, a pathological marker of AD, interacts with age to increase amyloid pathology in cognitively healthy and MCI subjects. This is consistent with the findings in our meta-analysis. Price & Morris (1999) also demonstrated an interaction between amyloid and NFTs. The deposition of large numbers of amyloid plaques is associated with both the number of NFTs and the rate of tangle formation with age (Price & Morris, 1999). We also found that the discrepancy in amyloid prevalence in subjects with or without NFTs decreased as age increased. A recent paper of Jack et al. (2014) indicates that amyloid deposition accelerates NFT accumulation, whereby amyloid pathology remains relatively stable at a given time and NFT neurodegeneration continuous to proceed. This supports the presumption that the prevalence of amyloid pathology interacts with NFTs.

In addition, amyloid pathology differed with age in subjects with or without diffuse plaques, independently of diagnosis. Diffuse plaques and amyloid/neuritic plaques together are mostly considered as the senile plaques occurring in the neuropathology of AD. Diffuse plaque development has been associated with normal aging, but also with the development of AD. Price et al., (2009) considered diffuse plaques as a prominent feature in the neuropathological diagnosis of AD in non-demented individuals. The association we found

between diffuse plaques and age is consistent with the enhanced amyloid pathology as an initial pathological event in the preclinical phase of AD.

Interestingly, we did not find an association between age at death and amyloid prevalence in our participant-level data. As age is considered as the single most important risk factor for de development of AD, we expected it to interact with amyloid pathology in the earlier stages of the disease. The finding is also in contrast with our finding in the meta-analysis, which showed an association between amyloid prevalence and age at death. One possible explanation for this contradictive finding is that most of the participants in our data ranged between an age of 84 and 91 ($n$=713), which contributes to not attending a significance level. We also found years of education not to be associated with amyloid prevalence. The mean years of education were 15 years (range 0-28). This indicates a relatively high educated subject group. Highly educated subjects are thought to have more effective brain networks, resulting in a greater capacity to maintain brain damage (Roe et al., 2007). These effective brain networks may lead to varying tolerance and speed of amyloid lesions and possibly allows for compensatory processes in undamaged networks. Assuming that highly educated individuals have an improved capacity to deal with brain damage, symptoms are not yet revealed at this stage of the disease and subjects remain cognitively normal for a longer period of time. In our meta-regression we also did not find an association between amyloid prevalence and years of education. This was presumably due to missing data on years of education in the meta-analysis.

*Limitations*

A first noteworthy limitation in our meta-analysis is the missing data on some variables. Only a few studies included had information on all, or even most, of the variables oriented. It should be taken into account that the establishment of associations for some variables were based on more participants, whereas the effects of other variables were based

on fewer participants. For instance, years of education was only available in five studies, whereas NFT staging was available in nine of the thirteen studies in the meta-analysis. This could ultimately have led to not identifying an association between amyloid prevalence and some variables. Another important limitation is that we not had subject-level data from the studies included in our meta-analysis. Despite the establishment of various predictive factors in the participant-level data from the NACC, it would have been useful to establish these predictive factors according to the data of the meta-analysis. Furthermore, analysis of subject-level data from our meta-analysis might not have led to contradictive findings in the associations between several risk factors for enhanced amyloid prevalence. Moreover, if we had these subject-level data, we could probably provide more generalizing conclusions.

*Conclusion and future directions*

A notable approach for future research is to estimate prevalence according to a combination of predictive factors from different behavioural and biological domains. To the extent that the variables included account as predictors in amyloid pathology, such a multivariate approach will increase overall prediction accuracy. Moreover, it also enables the investigation of possible interactive effects among various preclinical markers of AD.

Our paper provided further insight in the amyloid prevalence and the predictive factors of amyloid pathology in subjects without AD dementia. We determined which individuals are at increased risk for the development of AD. Assuming that individuals with amyloid pathology are on the path to AD, this study indicates that AD is a major health problem. We also established several predictive factors for amyloid pathology in the preclinical and prodromal stage of AD. Knowing who is at increased risk for future AD dementia leads to an easy and non-invasive way for screening individuals for participation in trials investigating effective disease-modifying treatments. Our research highlights the complex

nature of several risk factors on neuropathological aging and suggests a cooperating effect of neuropathological factors and genetic risk resulting in neurodegeneration.

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**Appendix 1**

**Study record form for meta-analysis 'prevalence abnormal amyloid'**

Site: Washington

Paper used as reference: Price et al. (2009). 'Neuropathology of non-demented aging: Presumptive evidence for preclinical Alzheimer disease.'

*Please check data already entered and complete empty fields if possible*

For any questions please contact Willemijn Jansen (willemijn.jansen@maastrichtuniversity.nl)

**1. Subject selection**

| | |
|---|---|
| Cohort | The National Alzheimer Coordinating Center-supported Neuropsychological Database Initiative. |

| | |
|---|---|
| Setting (clinical/research/population/mixed) | Research |

| | |
|---|---|
| Way of recruitment (random/consecutive patients/convenience sample/advertisements) | Random |

| | |
|---|---|
| Inclusion criteria | CDR = 0 (indicating the absence of dementia and mild cognitive impairment)<br>Autopsy within 2 years of death<br>≥ 60 years at death |

| | |
|---|---|
| Exclusion criteria | |

| | |
|---|---|
| Definition clinical diagnosis before death 'normal' | CDR = 0 |

| | |
|---|---|
| Definition clinical diagnosis before death 'MCI' | CDR > 0 |

Remarks: ……

**2. Neuropathological procedures**

| | |
|---|---|
| Neuropathological criteria used | Khachaturian<br>Washington University<br>CERAD<br>NIA-Reagan<br>Braak and Braak |

| | |
|---|---|
| Definition neuropathological Alzheimer | |

Remarks: ……

**3. Subject characteristics**

*Please check data already entered and complete empty fields if possible. You can use the subjects described in the reference above but you can also include other subjects if this increases the sample size.*

| ***Last clinical diagnosis before death:*** | ***Normal*** | ***MCI*** |
|---|---|---|
| **N** | 97 | 11 |
| **Mean age** | 84.3 | |
| **N female gender** | 55 | |
| **Mean education (years)** | 15.4 | |
| **Mean MMSE** | 28.1 | |
| **Mean CDR** | 0 | |

| | | |
|---|---|---|
| **Mean memory score *** | | |
| **N APOEe4 +** | | |
| **Interval between last clinical diagnosis and death (months)** | | |
| **N neuropathological diagnosis 'AD'** | Khachaturian: 46/97<br>Washington University: 38/97<br>CERAD: 38/97<br>NIA-Reagan: 38/97<br>Braak and Braak: Stage V = 3 | |
| **N neuropathological diagnosis 'no AD'** | Khachaturian: 51<br>Washington University: 59<br>CERAD: 59<br>NIA-Reagan: 59<br>Braak and Braak: Stage V = 94 | |

*Memory test used: ……

**4. Prevalence Alzheimer pathology**

*Please provide number of subjects in each category according to the neuropathological criteria you used or send data such that we can complete. Use definition for AD pathology according to the definition above. You can use the subjects described in the reference above but you can also include other subjects if this increases the sample size. Data according to age and APOE genotype can be completed in section 4.2.*

## 4.1 Prevalence Alzheimer pathology according to age

### CERAD neuritic plaque score

**Controls - all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuritic plaques* | 60-69 | 70-79 | 80-89 | >89 |
| Frequent or moderate | | | | |
| Sparse or no | | | | |

**MCI – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuritic plaques* | 60-69 | 70-79 | 80-89 | >89 |
| Frequent or moderate | | | | |
| Sparse or no | | | | |

### Braak & Braak neurofibrillary stage

**Controls – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Braak & Braak* | 60-69 | 70-79 | 80-89 | >89 |
| Stage 0-2 | | | | |
| Stage 3-6 | | | | |

**MCI – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Braak & Braak* | 60-69 | 70-79 | 80-89 | >89 |
| Stage 0-2 | | | | |
| Stage 3-6 | | | | |

**NIA/Reagan Institute neuropathological criteria**

**Controls – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

**MCI – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

**CERAD neuropathological criteria**

**Controls – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

**MCI – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

**Khachaturian neuropathological criteria**

**Controls – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

**MCI – all**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

**Other neuropathological criteria: ……**

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

### 4.2 Prevalence Alzheimer pathology according to age and APOE genotype

*Please provide number of subjects in each category or send data such that we can complete. Use definition for AD pathology according to the definition above.*

**Controls**

E4 Positive (at least 1 e4 allele)

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

E4 Negative

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

**MCI**

E4 Positive (at least 1 e4 allele)

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

E4 Negative

| N | *Age* | | | |
|---|---|---|---|---|
| *Neuropathological diagnosis* | 60-69 | 70-79 | 80-89 | >89 |
| AD | | | | |
| No AD | | | | |

Remarks: ……