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Prevalence of the Apolipoprotein E ε4 allele in amyloid-β positive subjects across the spectrum of Alzheimer’s disease

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Keywords:

APOE; Prevalence; Amyloid; PET; CSF; Alzheimer’s disease; Mild cognitive impairment; Subjective cognitive decline; Age; Sex; Education; Geographical location.

Highlights:

- Prevalence of APOE ε4 in Alzheimer’s disease has been underestimated in previous studies
- Prevalence of APOE ε4 decreases with age in early AD, but not at the dementia stage
- Prevalence of APOE ε4 is highest in Northern Europe
- Education and sex did not affect the prevalence of APOE ε4

Research in context:

1. **Systematic review**: Previous studies examining the prevalence of APOE ε4 in AD have included patients based on clinical criteria, without using biomarker information. This may have led to an underestimation of the prevalence of APOE ε4 due to misdiagnosis.

2. **Interpretation**: Our results demonstrate that PET or CSF evidence for the presence of amyloid-β is associated with a higher prevalence of APOE ε4 (66% versus 50-60 in previous studies).

3. **Future directions**: Information on APOE ε4 status would improve algorithms to determine risk for amyloid-β positivity, for example to enrich clinical trials. Furthermore, similar studies in amyloid-β positive subjects should be performed to determine the prevalence of other AD susceptibility genes.
ABSTRACT

INTRODUCTION: APOE ε4 is the major genetic risk factor for Alzheimer’s disease (AD), but its prevalence is unclear since earlier studies did not require biomarker evidence of amyloid-β (Aβ) pathology.

METHODS: We included 3,451 Aβ+ subjects (853 AD-type dementia, 1,810 mild cognitive impairment (MCI), and 788 cognitively normal (CN)). Generalized estimating equation models were used to assess APOE ε4 prevalence in relation to age, sex, education and geographical location.

RESULTS: The APOE ε4 prevalence was 66% in AD-type dementia, 64% in MCI and 51% in CN, and decreased with advancing age in Aβ+ CN and Aβ+ MCI (p<0.05), but not in Aβ+ AD dementia (p=0.66). The prevalence was highest in Northern Europe, but did not vary by sex or education.

DISCUSSION: The APOE ε4 prevalence in AD was higher than in previous studies, which did not require presence of Aβ pathology. Furthermore, our results highlight disease heterogeneity related to age and geographical location.
1. Introduction

Alzheimer’s disease (AD) is the most common type of dementia and a major cause of morbidity and mortality worldwide [1]. Pathological metabolism and accumulation of β-amyloid (Aβ) peptides is thought to be an initiating event in AD, leading to downstream spread of tau pathology, synaptic loss, neurodegeneration and cognitive decline [2-4]. The main risk factors for the development of AD are increasing age and the ε4 allele of the apolipoprotein E (APOE) gene [5-7], the strongest genetic risk factor for sporadic AD [8, 9]. APOE encodes for apolipoprotein E, which is a major lipid transporting protein in the brain [10]. In humans, the gene exists in three allele variants called ε2, ε3, and ε4. Compared to APOE ε3/ε3 (the most common genotype), APOE ε4 heterozygosity increases the risk for developing clinical AD by about 3-4 times, and APOE ε4 homozygosity by about 10-15 times [8, 11]. The overall prevalence of APOE ε4 positivity has been reported to be approximately 15-20% in the normal population [11, 12] and 50-60% in patients with AD dementia [8, 9, 13]. These numbers, however, vary widely and may depend on different characteristics of the study population, including ethnicity [14] and geographical location [13]. Additionally, most previous studies included clinically diagnosed AD patients, without neuropathological confirmation and/or supportive pathophysiological AD biomarkers. Studies applying cerebrospinal fluid (CSF) and positron emission tomography (PET) have revealed that a substantial proportion of patients with a clinical diagnosis of AD dementia have no evidence of Aβ-pathology [15-18], which makes underlying AD pathology highly unlikely. This mismatch between the clinical diagnosis and Aβ biomarkers seems especially prevalent in APOE ε4 non-carriers, as illustrated by a clinical trial in which 36% of APOE ε4-negative patients with a diagnosis of “AD dementia” lacked Aβ-pathology as
determined by PET [19]. Earlier studies emphasize the importance of the matter, as 
APOE ε4 was found to be more strongly associated with biomarker evidence of Aβ 
pathology (irrespective of clinical status) than a clinical diagnosis of AD [20]. Similarly, the effect size of APOE ε4 increased if presence or absence of Aβ 
pathology was neuropathologically confirmed [21].

Another critical point of previous studies is the focus on the dementia stage of AD. AD is believed to follow a long trajectory in which Aβ pathology is present and clinical symptoms gradually develop before the threshold for dementia is reached [22-24]. Few studies have investigated APOE ε4 positivity in prodromal AD[25], i.e. mild cognitive impairment (MCI) due to AD (Aβ-biomarker positive), but prevalence rates around 25-55% have been reported. Similarly, not many studies reported the proportion of APOE ε4 carriers among people with preclinical AD, i.e. presence of Aβ pathology without clinical symptoms [26-29].

In the present study, we aimed to investigate the prevalence of APOE ε4 positivity across the clinical and preclinical spectrum of AD in a large sample of Aβ-biomarker positive individuals, including cognitively normal controls (CN), MCI, and AD dementia. We also tested whether the prevalence of APOE ε4 positivity varied by age, sex and geographical location. For comparison, we included a group of Aβ-negative participants.
2. METHODS

2.1 Participants

We used data from the Amyloid Biomarker Study Group, which is a worldwide collaborative project on Aβ PET and CSF biomarkers in conjunction with demographic, clinical and genetic variables [5, 30, 31]. From all contributing sites, we received individual participant-level data on 9,480 individuals (3,903 CN, 4,189 MCI, 1,359 probable AD dementia and 538 non-AD dementia). Since we aimed to investigate the prevalence of APOE ε4 across the spectrum of AD, we applied the following selection procedure for this study: i) we excluded patients with a clinical diagnosis of non-AD dementia, ii) among CN, MCI or AD dementia participants, we selected Aβ-positive (Aβ+) individuals as determined by PET and/or CSF and their Aβ-negative (Aβ-) counterparts for comparison, and iii) we excluded individuals who lacked information on APOE ε4 status.

Normal cognition was defined as normal scores on cognitive tests, the absence of cognitive complaints (for which medical help was sought), or both [5, 31]. Some of the CN participants had subjective cognitive decline (SCD, n=533 [102 Aβ+, 431 Aβ-]), defined as presence of a cognitive complaint but normal cognition on neuropsychological tests [32]. We combined SCD subjects with the other CN [24, 33], except for one sub-analysis (paragraph 3.7). MCI and probable AD dementia were defined according to established diagnostic criteria [22, 23, 34]. Aβ- “AD dementia” cases most likely do not have AD as the underlying cause of their cognitive impairment, although it should be noted that Aβ biomarkers could misclassify subjects, especially when biomarker signals are close to the cut-offs [35, 36].
2.2 PET/CSF procedures

Individual PET scans were dichotomized (Aβ+ or Aβ-) using quantitative thresholds or visual reads according to the method used at the study site [5, 30]. CSF biomarkers were dichotomized as negative (normal) or positive (abnormal) using study-specific cutoffs [5]. For AD dementia patients we only had PET data available [30]. For NC and MCI, we selected the first available biomarker in time if a participant had both PET and CSF data [5]. Detailed PET/CSF procedures for each site are presented in Supplemental Table 1.

2.3 APOE genotyping

By design, all participants in this study had data on APOE ε4 status. For 2,955/3,114 (95.5%) CN and 3,054/3,335 (91.6%) MCI subjects we had specific genotypes (e.g. ε3/ε4, in addition to APOE ε4 status), which allowed breakdown into APOE ε4 non-carriers, heterozygotes and homozygotes. Specific genotypes were not available for AD dementia patients, as they were only collected for CN and MCI participants in our previous studies [5, 30].

2.4 Age, sex, education and geographical location

Information on age at time of clinical assessment was available for all participants. There were missing data for sex (130/7,419, 1.8%) and years of education (1,137/7,419, 15.3%). We used a previously published classification system for geographical location [13] to divide the participants into Southern Europe (n=653[215 Aβ+, 438 Aβ-]), Central Europe (n=832[343 Aβ+, 489 Aβ-]), Northern Europe
(n=1,667[792 Aβ+, 875 Aβ-]), Australia (n=395[190 Aβ+, 205 Aβ-]), North America (n=3,359[1,292 Aβ+, 2,067 Aβ-]) or Asia (n=315[114 Aβ+, 201 Aβ-]). Some participants (n=637[303 Aβ+, 334 Aβ-], 8.1%) could not be classified, as they were included in a multicenter study that covered multiple geographical locations.

2.5 Statistical analyses

Baseline differences were assessed using analysis of variance (with post-hoc Bonferroni correction) and χ² tests. The prevalence of APOE ε4-positivity was defined by calculating the percentage of APOE ε4-positive individuals of the total number of participants in each diagnostic group. Generalized estimating equations (GEE) were used to estimate the effects of age, sex, education and geographical location on the prevalence of APOE ε4-positivity. GEE was the method of choice for the study as it allows analysis of binary-correlated data, such that participant-level data from all cohorts can be modeled while simultaneously accounting for participants within studies. A logit link function for binary outcome with an exchangeable correlation structure was assumed to account for within-study correlation. Analyses were conducted using the total study population, unless specified otherwise. Age was entered as a continuous measure centered at the mean. We tested 2-way and 3-way interactions between variables, and these terms were retained in the model if they appeared significant by the Wald statistical test. The GEE derived unstandardized β-coefficients and standard errors (SE) of the main effect were reported. Significance was set at p<0.05 (two-sided). SPSS software (IBM, version 23.0) was used for statistics.
3. RESULTS

3.1 Participants

Demographic and clinical information for each diagnostic group is provided in Table 1. We included 7,419 subjects, among which 970 with a clinical diagnosis of AD dementia (853 Aβ+, 117 Aβ-), 3,335 with MCI (1,810 Aβ+, 1,525 Aβ-) and 3,114 CN subjects (788 Aβ+, 2,326 Aβ-). Demographic differences among the diagnostic groups included fewer males in the CN group (p<0.05) and less education in the MCI group compared to the other groups (p<0.001). Furthermore, in the dementia group Aβ-status was only determined using PET, while in the MCI group the proportion of subjects with CSF data (78%) was greater than that in the CN group (64.9%). In Aβ+ individuals, comparisons within diagnostic groups between APOE ε4 positive and negative groups showed that the mean age was lower in APOE ε4-positive than in APOE ε4-negative CN and MCI patients (p<0.01) (Supplemental Table 2). Supplemental Table 3 shows the demographic and clinical characteristics of individuals tested versus not tested for APOE in the complete Amyloid Biomarker Study Group dataset [5, 30, 31].

3.2 Prevalence of APOE ε4 positivity

In Aβ+ subjects, the prevalence of APOE ε4 positivity was 50.9% in CN, 63.5% in MCI and 66.1% in AD dementia (Table 1). The prevalence of APOE ε4-positivity was higher in Aβ+ MCI and Aβ+ AD dementia than in Aβ+ CN (p<0.001), but there was no difference between Aβ+ MCI and Aβ+ AD dementia (p=0.19). For comparison, the APOE ε4 prevalence in Aβ- subjects was 24.5% in CN, 27.9% in
MCI and 24.8% in AD dementia, which was significantly lower than in Aβ+ counterparts (all p<0.001).

3.3 Prevalence of APOE ε4 positivity by age, sex, education and modality

The prevalence of APOE ε4 positivity was lower at older age in Aβ+ CN (β for change in prevalence per year±standard error: -0.02±0.01, p<0.05, Figure 1) and Aβ+ MCI (β=-0.03±0.01, p<0.01). For example, at age 50, the prevalence of APOE ε4 positivity was 61% in Aβ+ CN and 75% in Aβ+ MCI, compared to 42% and 47% at age 90, respectively (Supplemental Figure 1). There was no age effect in AD dementia (β=0.01±0.01, p=0.66). There was also no effect of age in AD dementia when excluding patients (n=91) with a known atypical presentation, which are typically associated with lower prevalence of APOE ε4 (β=0.00±0.01, p=0.99, Supplemental Figure 2). In Aβ- subjects, the prevalence of APOE ε4 also decreased with age in CN (β=-0.03±0.01, p<0.001; difference with Aβ+: p=0.62) and MCI (β=-0.03±0.01, p<0.001; difference with Aβ-: p=0.82), but not in AD dementia (β=-0.01±0.02, p=0.55; difference with Aβ+: p=0.19)). All effects described above were similar when adjusting for sex and education.

In Aβ+ subjects, sex and education had no direct effects on APOE ε4 positivity, either across or within diagnostic groups (all p>0.05). Furthermore, in Aβ+ subjects there was an interaction between age and sex (p<0.05), whereby prevalence decreased with age for women but not for men. Examining the three-way interaction with diagnosis revealed that the interaction between age and sex was present in MCI (p<0.01), and at trend level in AD dementia (p=0.053), but not in CN subjects.
(p=0.26). In Aβ- MCI subjects, there was a trend towards greater prevalence of APOE ε4 positivity in women (β: 0.19±0.10, p=0.06). There were no direct of interaction effects for education and no interaction effects (all p>0.05). The prevalence of APOE ε4 positivity was higher for CSF than for PET only in Aβ- MCI subjects ($\chi^2 = 6.68$, p=0.01; Supplemental Table 4). See Supplemental Table 5 for an overview of all main and interaction effects.

### 3.4 Prevalence of specific APOE genotypes in CN and MCI

Next, we stratified CN (n=2,955 [751 Aβ+, 2,204 Aβ-]) and MCI (n=3,054 [1,638 Aβ+, 1,416 Aβ-]) subjects with APOE genotype information available into groups of APOE ε4 non-carriers, APOE ε4 heterozygotes and APOE ε4 homozygotes, and divided them into quartiles according to age. Both in CN and MCI the proportion of APOE ε4 heterozygotes and APOE ε4 homozygotes decreased with advancing age (Figure 2). Prevalence of the specific genotypes (i.e. APOE ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4) is provided in Table 2.

### 3.5 Prevalence of APOE ε4 positivity by geographical location

Next, we assessed the effect of geographical location on prevalence of APOE ε4 positivity. Within Aβ+ subjects, we found that the prevalence of APOE ε4 positivity across diagnostic groups was higher in Northern Europe compared to all other geographical locations except Australia (all p<0.001, Bonferroni corrected; Figure 3A). In addition, the prevalence of APOE ε4 positivity was lower in Southern Europe compared to North America, Central Europe (p<0.05, uncorrected) and Australia.
(p<0.001, Bonferroni-corrected), and higher in Australia than in Asia (p<0.05, uncorrected). Within Aβ- subjects, the prevalence of APOE ε4 positivity was higher in Northern Europe (p<0.001, Bonferroni-corrected) and Central Europe (p<0.05, uncorrected) compared to all other geographical locations (Figure 3B). These findings were similar when assessing each diagnostic group separately (Supplemental Figure 3, Supplemental Table 5).

3.6 Predictive effect of APOE ε4 status on disease stage

Finally, to assess whether the APOE allele is predictive of AD dementia or MCI beyond its effect on Aβ, we performed binary logistic regression models, including age, sex, education, Aβ status (positive/negative) and APOE ε4 status (positive/negative) for CN vs MCI and CN vs AD. We found that APOE ε4 status predicted both CN vs MCI (odds ratio [OR]: 1.629, 95% confidence interval [CI]: 1.348-1.968, p<0.001) and CN vs AD (OR: 1.811, 95% CI: 1.457-2.251, p<0.001).

3.7 Prevalence of APOE ε4 positivity by subjective cognitive decline

The prevalence of APOE ε4 was higher in participants with SCD compared to those without, both among Aβ+ (64.7% vs 48.8%, p<0.05) and Aβ- (33.6% vs 22.4%, p<0.05) subjects (Supplemental Table 6). The relationship between age and APOE prevalence was not affected by the presence or absence of SCD (all p<0.05).
4. Discussion

We found that the prevalence of $APOE\,\varepsilon4$ positivity was 51% in preclinical AD (Aβ+ CN), 64% in prodromal AD (Aβ+ MCI) and 66 % in Aβ+ AD dementia. Among Aβ-subjects the prevalence of $APOE\,\varepsilon4$ positivity was 25% in CN, 28% in MCI and 25% in AD dementia. Our estimates of $APOE\,\varepsilon4$ prevalence in Aβ-biomarker verified AD-type dementia are higher than reported in previous studies that defined AD-type dementia based on clinical criteria. This resonates well with studies examining the effect size of $APOE\,\varepsilon4$ in pathology- or biomarker-confirmed cases [20, 21] and suggests that the prevalence of $APOE\,\varepsilon4$ in AD-type dementia (66%) may have been underestimated in previous studies (50-60% [8, 9, 13]).

Another main finding of this study was that the prevalence of $APOE\,\varepsilon4$ decreased with age in preclinical and prodromal AD. There are several possible explanations. First, the additive effects of $APOE\,\varepsilon4$ and Aβ may have resulted in greater conversion from the CN and MCI groups to AD dementia.[37] Higher conversion rates could also be due to earlier and more pronounced accumulation of Aβ load in $APOE\,\varepsilon4$ carriers [38], but the binary nature (Aβ positive or negative) of our dataset does not allow testing of this hypothesis. Second, supposedly due to the increased risk for cardiovascular diseases in ε4 carriers, $APOE\,\varepsilon4$ has been linked to increased mortality rates [39-41]. This observation fits our finding that $APOE\,\varepsilon4$ carriership also decreased with age in Aβ- CN and MCI subjects, although the reduction of $APOE\,\varepsilon4$ in Aβ- subjects can also be caused by individuals transitioning from Aβ- to Aβ+ with advancing age. Finally, as $APOE\,\varepsilon4$ accelerates the onset of amyloid aggregation by approximately 15 years [5, 26], the prevalence of ε4 carriers in Aβ+ subjects will be higher at younger age ranges. Remarkably, the prevalence of
APOE ε4 did not change with age in AD-type dementia. It may be hypothesized that the higher mortality in APOE ε4 carriers is counterbalanced at the dementia stage by individuals transitioning from preclinical and prodromal AD into AD dementia. We also tested whether this lack of an age effect was caused by the inclusion of atypical variants of AD dementia as this group is characterized by lower prevalence of APOE ε4 [42, 43], but this was not the case (Supplementary Figure 2). The pathogenesis of early-onset AD is complex since this group includes a mix of APOE ε4-carriers who develop the disease at younger age and of APOE ε4 non-carriers with rapidly progressive AD [44, 45]. This may confound relationships between APOE ε4 and age, especially in young patients with AD-type dementia. Furthermore, it has been shown that the mortality effect of APOE ε4 is less pronounced at older age [46], which may explain the lack of an age effect in AD dementia patients. It is not clear why Aβ+ women had decreasing prevalence of APOE ε4 with age. However, a recent large meta-analysis also found an interaction between APOE ε4, sex and age, so that APOE ε4 conferred a greater risk for AD in women than in men at younger ages, but not in older [47]. It is possible that physiological changes around menopause may interact with APOE ε4 in women and increase the risk for Aβ pathology in younger ages [48]. If this leads to an earlier onset of the disease, and earlier death, the APOE ε4 prevalence may appear to decrease with age in Aβ+ women.

Another main finding was the lower prevalence of APOE ε4 in both Aβ+ and Aβ- CN subjects compared to the MCI and dementia stages. This may be explained by a selection bias, as the vast majority of the MCI and AD dementia subjects visited a memory clinic, while many CN subjects were recruited as research volunteers. Also, APOE ε4+ MCI patients may be more likely to seek medical help and APOE ε4
carriers with dementia may be more willing to participate in research due to a positive family history. Another possible reason is that APOE ε4 may accelerate the transition from preclinical to clinical AD. For example, APOE ε4 may have an effect on brain structure and function through non-Aβ pathways [49-53], which may act synergistically with Aβ-pathology to shorten the time between start of Aβ deposition and cognitive decline. Thus, since APOE ε4 carriers will develop symptoms earlier, prevalence of APOE ε4 positivity in CN is lower than in MCI and dementia cases at the same age range. Finally, APOE ε4 non-carriers (which would include APOE ε2 carriers) may have mechanisms of resilience (i.e., cognitive reserve) that are less present in ε4 carriers [54].

We also found geographical differences in APOE ε4 prevalence, with higher prevalence in AD patients from Northern Europe, Central Europe, and Australia, and lower prevalence in patients from Southern Europe and Asia. This is consistent with previous epidemiological studies in clinically diagnosed AD dementia and MCI patients [13, 55] and with lower prevalence of APOE ε4 in the general population in Southern Europe and Asia compared to Northern Europe [14, 55-57]. The novelty of this study is that we confirm these geographical differences in Aβ-biomarker defined AD and throughout the continuum from preclinical to prodromal and dementia stages. The different geographical prevalence of APOE ε4 may be important for recruitment of participants in clinical trials and for the use of APOE ε4 in algorithms to predict Aβ-positivity [58].

Strengths of this study include the large number of Aβ-positive subjects across the spectrum from preclinical to prodromal and dementia stages of AD. Limitations include that relatively few participants came from Asia (n=315) and Australia
(n=394), and there were no participants from Africa and South America. There were no data on ethnicity of the participants, which may confound the results since ethnicity has been related to both APOE ε4 and AD [14, 59]. Also, this study is based on an assembly of different study cohorts that may not be representative for typical memory clinic populations or the general population. Finally, Aβ-positivity was determined using different modalities (i.e. PET or CSF) and methods (e.g. visual read versus quantitative threshold for PET and different assays for CSF). There was an unexpected effect of CSF assay (Innotest vs Luminex), which could be interpreted as a cohort effect as the majority of subjects with CSF analyzed using the Luminex assay are ADNI participants (Supplemental Table 5). We found no effects of modality (PET vs CSF) on APOE ε4 prevalence, and in previous studies using these data we found only little evidence for heterogeneity related to modality and methodology [5, 30].

With about 2/3 of prodromal AD and AD dementia patients being APOE ε4 carriers, our results further emphasize the importance of APOE ε4 for the development of AD [8, 9]. This may be useful for development of disease-modifying treatments, which may be focused on attenuating the detrimental effects of APOE ε4, and for understanding the molecular pathogenesis of AD [60]. Furthermore, the finding that the prevalence of APOE ε4 decreases with age in CN and MCI subjects has potential implications for clinical trials in pre-dementia populations, as screening based on APOE status to enrich for Aβ-positivity may be less effective with advancing age. Last, it may be of importance to evaluate other proposed AD susceptibility genes [61] in cohorts with known Aβ status, as to date this has only
been assessed in cohorts of clinically diagnosed AD patients and cognitively normal elderly.

5. Conclusions

We have quantified the prevalence of APOE ε4 in Aβ-biomarker defined preclinical AD, prodromal AD and AD dementia. The results emphasize the prominent role of APOE ε4 in AD, but also point to disease heterogeneity, since APOE ε4 positivity is markedly less common in elderly subjects in pre-dementia stages of AD and in people from specific geographical locations, including Southern Europe and Asia. Further studies on phenotypic differences between APOE ε4-negative and APOE ε4-positive AD patients may be important to understanding different pathways that may lead to AD, and ultimately to tailor disease-modifying treatments to specific patient subgroups.
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Disclosures

Dr Aarsland reported having received research support or honoraria from AstraZeneca, H. Lundbeck, Novartis Pharmaceuticals, and GE Health. Dr Anders Wallin reported having received speakers’ bureau fees from Esai and Triolab and serving on the advisory board for Nutricia and Esai. Dr Blennow reported having received personal fees (advisory boards or consulting) from Roche Diagnostics, IBL International, Novartis, Fujirebio Europe, and Eli Lilly and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. Dr Chen reported having received grants from the National Institutes of Health (NIH). Dr Drzezga reported having received speaker honoraria and consulting fees from GE Healthcare, AVID/Lilly, and Piramal. Dr
Fagan reported having received grants from NIH, Fred Simmons and Olga Mohan, and Charles and Joanne Knight Alzheimer’s Research Initiative of the Washington University Knight Alzheimer’s Disease Research Center; having received personal fees (advisory boards or consulting) from IBL International, Roche Diagnostic, Diamir, and AbbVie. Dr Fladby reported having a patent “Methods and compositions for monitoring phagocytic activity,” PCT/US2011/062233, pending. Dr Fleisher reported having been a full-time employee of the Banner Alzheimer’s Institute at the time of data collection; currently being a full-time employee of Eli Lilly. Dr Förster reported having received personal fees (consultancy) from Piramal, Bayer, and GE. Dr Frisoni reported having received grants and/or personal fees from Lilly, Bristol-Myers Squibb, Bayer, Lundbeck, Elan, AstraZeneca, Pfizer, Taurx, Wyeth, GE, Baxter, Avid, Roche, Piramal, and the Alzheimer’s Association. Dr Gill reported having received grants from the Indian Council of Medical Research, New Delhi, India. Dr. Grimmer reported having received consulting fees from Actelion, Eli Lilly, MSD, Novartis, Quintiles, Roche Pharma, lecture fees from Biogen, Lilly, Parexel, Roche Pharma and grants to his institution from Actelion and PreDemTech. Dr. Harald Hampel declares no conflict of interest with the content of the present manuscript. He serves as Senior Associate Editor for the Journal Alzheimer’s & Dementia; he has been a scientific consultant and/or speaker and/or attended scientific advisory boards of Axovant, Anavex, Eli Lilly and company, GE Healthcare, Cytox Ltd, Jung Diagnostics GmbH, Roche, Biogen Idec, Takeda-Zinfandel, Oryzon Genomics, Qynapse; and he receives research support from the Association for Alzheimer Research (Paris), Pierre and Marie Curie University (Paris), Pfizer & Avid (paid to institution); and he has patents, but receives no royalties. Dr Hansson has received research support (to the institute) from GE Healthcare, AVID radiopharmaceuticals
and Hoffmann-La Roche. Dr Jagust reported having received personal fees from Banner Alzheimer Institute/Genentech, Synarc, Biogen and Novartis. Dr Ivanoiu reported having served on an advisory board for Eli Lilly and Nutricia, having received compensation as a speaker and consultant for GE Healthcare and Nutricia, having received clinical trial agreements with GEHC, Merck and Eli Lilly, having received grants from the Fonds de la Recherche Scientifique (F.R.S.–FNRS), Belgium and non-financial support from GEHC. Dr Jansen reported having received research support from Biogen. Dr Klunk reported being a co-inventor of the amyloid imaging tracer PiB and, as such, having a financial interest in the license agreement. (PiB intellectual property is owned by the University of Pittsburgh, and GE Healthcare holds a license agreement with the University of Pittsburgh based on the PiB technology described in this article and receives “inventors share” payments from the University of Pittsburgh based on income from that license.). Dr Kornhuber reported having received grants from German Federal Ministry of Education and Research (BMBF): Kompetenznetz Demenzen (01GI0420) and German Federal Ministry of Education and Research (BMBF): The Frontotemporo-Lobar Degeneration Consortium (FTDL-C), 01GI1007A and having a patent, PCT/EP2004/003963, “Diagnosis of Alzheimer’s disease,” issued; a patent, EP 1811304 A1, “Large Aβ-peptide binding particles (LAPS) in diagnosis and therapy of Alzheimer’s dementia,” issued; a patent, WO2007/082750 A1, “Immunoglobulin- bound Ab-peptides and immunoglobulins-binding Ab-peptides in diagnosis and therapy of Alzheimer’s dementia,” issued; a patent, EP 2437067A2, “Methods of differentially diagnosing dementias,” issued; and a patent, “New formulations for diagnosis of Alzheimer’s disease,” pending. Dr Landau reported having received grants from NIH and personal fees from Biogen Idec, Genentech, and Synarc. Dr Lleo reported having received
grants from Instituto de Salud Carlos III (Fondo de Investigación Sanitario, PI10/01878; PI13/01532; PI11/2425; PI11/3035 and the CIBERNED program). Dr Mintun reported being an employee of Avid Radiopharmaceuticals, a wholly owned subsidiary of Eli Lilly. Dr Morris reported having received grants from NIH (P50AG005681, P01AG003991, P01AG026276, U19AG032438). Dr Mroczko reported having received grants and personal fees from the Leading National Research Centre (KNOW), Medical University of Bialystok, Poland; and consultation and/or lecture honoraria from Roche, Cormay and Biameditek. Dr Peters reported having received grants and/or personal fees from Lilly, Roche, Genentech, Lundbeck, Affiris, Piramal, Novartis, and Trx-Pharmaceuticals. Dr Popp reported having received grants from the Swiss National Science Foundation (SNF 320030L_141179), Fujirebio Europe, and from the Nestlé Institute of Health Sciences. Dr Rabinovici reported having received grants from Avid Radiopharmaceuticals and personal fees from GE Healthcare and Piramal. Dr Rinne reported having received grants from Sigrid Juselius Foundation and Turku University Hospital clinical grants. Dr Rowe reported having received grants from Avid Radiopharmaceuticals, Piramal Imaging, AstraZeneca, GE Healthcare, Avid/Lilly, Navidea, CSIRO, NHMRC, Alzheimer’s Association, and an anonymous foundation and having had a patent licensed for PET image processing. Dr Sarazin reported having received personal fees from Eisai, Janssen, Novartis (lecture) and Allianz (lecture), and research grants from the French Health Ministry, Institute Roche de Reserche et Médecine Translationelle (paid to the institution). Dr Scheltens reported having received grants from GE Healthcare, Piramal, and Merck, paid to his institution. Dr Soininen reported having received grants from the Academy of Finland, European Union 7ThFP 601055 VPH-DARE, Kuopio University Hospital VTR, and University of Eastern Finland. Dr Teunissen
reported being a member of the international advisory board at Innogenetics and Roche; and having research contracts at Probiodrug, Boehringer, Roche, EIP Pharma, Brainsonline, AxonNeurosciences and PeopleBio. Dr van der Flier reported having received grants from Boehringer Ingelheim, Piramal Imaging, and Roche. Dr Van Laere reported having received grants through KU Leuven from Merck, Janssen Pharmaceuticals, UCB, Novartis, Pfizer, and GE Healthcare. Dr Vandenberghe reported having received clinical trial agreements with GEHC, Merck, Forum, and Roche; grants from Research Foundation–Flanders (FWO) and KU Leuven; and nonfinancial support from GEHC. Dr Verbeek reported having served on an advisory board for Roche. Dr Verhey reported having received compensation as a speaker and consultant for Nutricia Advanced Medical Food. Dr Visser reported having received research support from Biogen, grants from EU/EFPIA Innovative Medicines Initiative Joint Undertaking, EU Joint Programme–Neurodegenerative Disease Research (JPND), ZonMw, and Bristol-Myers Squibb; having served as member of the advisory board of Roche Diagnostics; and having received nonfinancial support from GE Healthcare. Dr Vos receives research support from Janssen Pharmaceutica N.V. and grants from ZonMw and EU/EFPIA Innovative Medicines Initiative Joint Undertaking. Dr Waldemar reported being a board member of the Lundbeck Foundation. Dr Wolk reported having received personal fees from GE Healthcare and Piramal Pharma and grants from Avid Radiopharmaceuticals. Dr Zetterberg is co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. The authors received compensation (ie, salary) as employees of their respective organizations. No other disclosures were reported.
Table 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>CN</th>
<th>MCI</th>
<th>AD dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Aβ-</td>
<td>Aβ+</td>
</tr>
<tr>
<td>N</td>
<td>3,552</td>
<td>2,764</td>
<td>788</td>
</tr>
<tr>
<td>Agea, mean</td>
<td>67.3±11.8</td>
<td>65.8±12.0</td>
<td>72.6±9.4</td>
</tr>
<tr>
<td>Age, range</td>
<td>18-109</td>
<td>18-93</td>
<td>32-109</td>
</tr>
<tr>
<td>Sexb (% male)</td>
<td>43.9</td>
<td>42.9</td>
<td>47.2</td>
</tr>
<tr>
<td>MMSEc, mean</td>
<td>29.0±1.2</td>
<td>29.0±1.2</td>
<td>28.8±1.3</td>
</tr>
<tr>
<td>Educationd, yrs</td>
<td>14.3±3.7</td>
<td>14.3±3.7</td>
<td>14.3±3.8</td>
</tr>
<tr>
<td>Modality for Aβ positivitye (% PET vs % CSF)</td>
<td>41.6/58.4</td>
<td>42.9/57.1</td>
<td>36.1/63.9</td>
</tr>
<tr>
<td>APOE ε4 positivityf (%)</td>
<td>30.5</td>
<td>24.6</td>
<td>50.9</td>
</tr>
</tbody>
</table>

Region:

North America, n 1,469 1,044 425 1,077 412 665 375 50 325
<table>
<thead>
<tr>
<th>% APOE ε4 positive</th>
<th>432 (29.4)</th>
<th>238 (22.8)</th>
<th>194 (45.6)</th>
<th>522 (48.5)</th>
<th>96 (23.3)</th>
<th>426 (64.1)</th>
<th>227 (60.5)</th>
<th>7 (14)</th>
<th>220 (67.7)</th>
</tr>
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<tbody>
<tr>
<td>Australia, n</td>
<td>200</td>
<td>140</td>
<td>60</td>
<td>76</td>
<td>26</td>
<td>50</td>
<td>118</td>
<td>4</td>
<td>114</td>
</tr>
<tr>
<td>% APOE ε4 positive</td>
<td>76 (38)</td>
<td>38 (27.1)</td>
<td>38 (63.3)</td>
<td>42 (55.3)</td>
<td>4 (15.4)</td>
<td>38 (76.0)</td>
<td>72 (61.0)</td>
<td>-</td>
<td>72 (63.2)</td>
</tr>
<tr>
<td>Northern Europe, n</td>
<td>712</td>
<td>568</td>
<td>144</td>
<td>714</td>
<td>365</td>
<td>349</td>
<td>241</td>
<td>38</td>
<td>203</td>
</tr>
<tr>
<td>% APOE ε4 positive</td>
<td>251 (35.3)</td>
<td>164 (28.9)</td>
<td>87 (60.4)</td>
<td>375 (52.5)</td>
<td>125 (34.2)</td>
<td>250 (71.6)</td>
<td>166 (68.9)</td>
<td>16 (42.1)</td>
<td>150 (73.9)</td>
</tr>
<tr>
<td>Central Europe, n</td>
<td>195</td>
<td>154</td>
<td>41</td>
<td>536</td>
<td>304</td>
<td>232</td>
<td>101</td>
<td>12</td>
<td>89</td>
</tr>
<tr>
<td>% APOE ε4 positive</td>
<td>60 (30.8)</td>
<td>36 (23.4)</td>
<td>24 (58.5)</td>
<td>223 (41.6)</td>
<td>92 (30.3)</td>
<td>131 (56.5)</td>
<td>60 (59.4)</td>
<td>2 (16.7)</td>
<td>58 (65.2)</td>
</tr>
<tr>
<td>Southern Europe, n</td>
<td>269</td>
<td>221</td>
<td>48</td>
<td>343</td>
<td>163</td>
<td>180</td>
<td>41</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>% APOE ε4 positive</td>
<td>61 (22.7)</td>
<td>43 (19.5)</td>
<td>18 (37.5)</td>
<td>135 (39.4)</td>
<td>37 (22.7)</td>
<td>98 (54.4)</td>
<td>19 (46.3)</td>
<td>0 (0)</td>
<td>19 (47.5)</td>
</tr>
<tr>
<td>Asia, n</td>
<td>80</td>
<td>71</td>
<td>9</td>
<td>141</td>
<td>76</td>
<td>65</td>
<td>94</td>
<td>12</td>
<td>82</td>
</tr>
<tr>
<td>% APOE ε4 positive</td>
<td>18 (22.5)</td>
<td>14 (19.7)</td>
<td>4 (44.4)</td>
<td>47 (33.3)</td>
<td>10 (13.2)</td>
<td>37 (56.9)</td>
<td>49 (52.1)</td>
<td>4 (33.3)</td>
<td>45 (54.9)</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD unless indicated otherwise. Differences between diagnostic groups (assessed separately for Aβ-positive and Aβ-negative groups) were assessed using ANOVA (age, education, MMSE) and χ² tests (sex, modality and APOE ε4 status) with post hoc Bonferroni tests.

a Aβ- CN < MCI/AD, p<0.001, MCI < AD, p<0.01; Aβ+ CN/MCI > AD dementia, p<0.001
b Aβ- CN < MCI/AD, p<0.05; Aβ+ CN > MCI/AD dementia, p<0.05
c Aβ- CN < MCI/AD, p<0.001, MCI < AD, p<0.05; Aβ+ AD dementia < CN/MCI, p<0.001, MCI < CN, p<0.001
d Aβ- MCI < CN/AD, p<0.001; Aβ+ MCI < CN/AD dementia, p<0.001
e Aβ- AD > MCI/CN, CN > MCI, p<0.001; Aβ+ AD dementia > CN/MCI, p<0.001; CN > MCI, p<0.001
f Aβ+ AD dementia/MCI > CN, p<0.001

Aβ = amyloid-β, CN = cognitively normal, MCI = mild cognitive impairment, AD = Alzheimer’s disease; MMSE = Mini-Mental state examination; PET = Positron emission tomography; CSF = Cerebrospinal fluid; APOE = Apolipoprotein E.
Table 2. Prevalence of APOE genotype in cognitively normal and MCI subjects according to Aβ status

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Aβ +/- CN &amp; MCI, n (%)</td>
<td>22 (0.4)</td>
<td>566 (9.4)</td>
<td>126 (2.1)</td>
<td>3028 (50.4)</td>
<td>1845 (30.7)</td>
<td>422 (7.0)</td>
<td>714 (11.9)</td>
<td>5565 (92.6)</td>
<td>6,009 (37.7)</td>
<td>440 (6.8)</td>
</tr>
<tr>
<td>Aβ + CN &amp; MCI, n (%)</td>
<td>2 (0.1)</td>
<td>88 (3.7)</td>
<td>61 (2.6)</td>
<td>861 (36.0)</td>
<td>1027 (43.0)</td>
<td>350 (14.7)</td>
<td>151 (6.3)</td>
<td>2037 (85.3)</td>
<td>1,377 (57.6)</td>
<td>209 (8.0)</td>
</tr>
<tr>
<td>Aβ - CN &amp; MCI, n (%)</td>
<td>20 (0.6)</td>
<td>478 (13.2)</td>
<td>65 (1.8)</td>
<td>2167 (59.9)</td>
<td>818 (22.6)</td>
<td>72 (2.0)</td>
<td>563 (15.6)</td>
<td>3528 (97.5)</td>
<td>890 (24.6)</td>
<td>231 (6.0)</td>
</tr>
<tr>
<td>Aβ + CN, n (%)</td>
<td>1 (0.1)</td>
<td>28 (3.7)</td>
<td>19 (2.5)</td>
<td>336 (44.7)</td>
<td>304 (40.5)</td>
<td>63 (8.4)</td>
<td>48 (6.4)</td>
<td>687 (91.5)</td>
<td>367 (48.9)</td>
<td>37 (4.7)</td>
</tr>
<tr>
<td>Aβ + MCI, n (%)</td>
<td>1 (0.1)</td>
<td>60 (3.7)</td>
<td>42 (2.6)</td>
<td>525 (32.1)</td>
<td>723 (44.1)</td>
<td>287 (17.5)</td>
<td>103 (6.3)</td>
<td>1350 (82.4)</td>
<td>1,010 (61.7)</td>
<td>172 (9.5)</td>
</tr>
<tr>
<td>Aβ - CN, n (%)</td>
<td>15 (0.7)</td>
<td>311 (14.1)</td>
<td>38 (1.7)</td>
<td>1331 (60.4)</td>
<td>478 (21.7)</td>
<td>31 (1.4)</td>
<td>364 (16.5)</td>
<td>2158 (97.9)</td>
<td>509 (23.1)</td>
<td>122 (5.2)</td>
</tr>
<tr>
<td>Aβ - MCI, n (%)</td>
<td>5 (0.4)</td>
<td>167 (11.8)</td>
<td>27 (1.9)</td>
<td>836 (59.0)</td>
<td>340 (24.0)</td>
<td>41 (2.9)</td>
<td>199 (14.1)</td>
<td>1370 (96.8)</td>
<td>381 (26.9)</td>
<td>109 (7.1)</td>
</tr>
</tbody>
</table>

Information on APOE genotype was available in 93.2% of subjects with normal cognition and mild cognitive impairment. For subjects with AD dementia, only information on APOE status (+ or -) was provided.

Aβ = amyloid-β, CN = cognitively normal, MCI = mild cognitive impairment, APOE = Apolipoprotein E.
FIGURE LEGENDS:

Figure 1. Prevalence of \textit{APOE} $\varepsilon$4 positivity by age, diagnosis and A$\beta$ status

Curves were plotted using the point estimates generated by generalized estimating equations and are within the age limits of the diagnostic groups. The models were adjusted for study (site) effect. The 95% confidence intervals are presented in eFigure 1 in the Supplement.

AD = Alzheimer’s disease; \textit{APOE} = Apolipoprotein E

Figure 2. Distribution of \textit{APOE} $\varepsilon$4 negative, \textit{APOE} $\varepsilon$4 heterozygous and \textit{APOE} $\varepsilon$4 homozygous subjects across different age quartiles (Fig-2A; Q1= <67 years, Q2= 67-73.2, Q3= 73.21-78.76, Q4= >78.77 years: Fig-2B; Q1= <66.67 years, Q2= 66.68-72.28, Q3= 72.29-77.19, Q4= >77.2: Fig-2C; Q1= <59.5 years, Q2= 59.5-67.1, Q3= 67.11-75.65, Q4= >75.66 years; Fig-2D; Q1=<62 years, Q2= 62.01-68.41, Q3= 68.42-75.0, Q4= >75.01 years).

A$\beta$ = Amyloid-beta; \textit{APOE} = Apolipoprotein E; Q = Quartile.

Figure 3. Distribution of \textit{APOE} $\varepsilon$4 negative and \textit{APOE} $\varepsilon$4 positive subjects by geographical location for all A$\beta$+ (A) and A$\beta$- (B) participants across diagnostic groups. A further breakdown into diagnostic groups is provided in eFigure 2 in the Supplement. 8.1% of participants (n=637 [303 A$\beta$+, 334 A$\beta$-]) could not be classified, as they were included in a multicenter study that covered multiple geographical locations.

A$\beta$ = Amyloid-beta; \textit{APOE} = Apolipoprotein E
REFERENCES:


