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Alzheimers Dement. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

Alzheimers Dement. 2023 October ; 19(10): 4507–4519. doi:10.1002/alz.12986.

Plasma biomarkers identify older adults at risk of Alzheimer's disease and related dementias in a real-world population-based cohort

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Conflicts of interest and Disclosure Statement

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers. MG has given lectures at University of Connecticut and is Associate Editor honorarium at Journal of the American Geriatrics Society. PCLF, YZ, BS, C-CHC, BB, EJ, MIK, TAP, VLV, TKK reports no disclosures.

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Abstract

INTRODUCTION: Plasma biomarkers – cost-effective, non-invasive indicators of Alzheimer's disease and related disorders (ADRD) – have largely been studied in clinical research settings. Here, we examined plasma biomarker profiles and their associated factors in a population-based cohort to determine whether they could identify an at-risk group, independently of brain and CSF biomarkers.

METHODS: We measured plasma phosphorylated-tau181(p-tau181), neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and amyloid-β (Aβ)42/40 ratio in 847 participants from a population-based cohort in southwestern Pennsylvania.

RESULTS: K-medoids clustering identified two distinct plasma Aβ42/40 modes, further categorizable into three biomarker profile groups: *normal*, *uncertain* and *abnormal*. In different groups, plasma p-tau181, NfL and GFAP were inversely correlated with Aβ42/40, Clinical Dementia Rating, and memory composite score, with the strongest associations in the *abnormal* group.

DISCUSSION: *Abnormal* plasma Aβ42/40 ratio identified older-adult groups with lower memory scores, higher dementia risks and higher ADRD biomarker levels, with potential implications for population screening.

Keywords

Plasma ADRD biomarkers; Monongahela-Youghiogheny Healthy Aging Team (MYHAT); cognitive impairment; cluster modeling; aging; epidemiology

1. Introduction

Alzheimer's disease (AD) is characterized by brain deposition of amyloid-β (Aβ) plaques and tau neurofibrillary tangles[1]. Additional pathophysiological features include neurodegeneration/axonal damage and glial activation[2, 3]. While brain Aβ, tau, neurodegeneration and glial activation are quantifiable *in vivo* using established neuroimaging and cerebrospinal fluid (CSF) biomarkers[4–6], their prohibitive costs and limited availability hinder population-level applications[7]. Plasma biomarkers are accurate, more accessible, and cost-effective methods that can circumvent these limitations[7]. Multiple independent studies have demonstrated that plasma Aβ42/40 and p-tau181 are associated with brain Aβ and tau[8 9–14]. Furthermore, plasma neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) associate with brain degeneration and glial activation, respectively, which are found in both AD and related neurodegenerative disorders (ADRDs)[3, 12, 15, 16]. Nonetheless, previous investigations were limited mostly to clinical research cohorts with CSF/neuroimaging biomarkers categorization and also lacked diversity/heterogeneity in terms of social, economic and geographic origins[7, 17]. It is essential to assess plasma biomarker performance in population-based cohorts to: 1) verify their utility in less homogeneous groups of older adults[18], 2) understand biomarker

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associations with cognitive impairment and demographic characteristics, and 3) ascertain the potential generalizability of results documented in earlier studies.

Plasma biomarkers will be pivotal in community screening to identify at-risk individuals[7]. There is general agreement that plasma biomarkers will be pivotal in community screening to identify at-risk individuals. However, there is as yet no identified strategy for doing so. In this study, we investigated plasma Aβ42/Aβ40 ratio, p-tau181, GFAP, and NfL in a population-based cohort of older adults from medically under-served small towns of relatively low socio-economic status. We subsequently applied a novel clustering approach to categorize the participants into groups of distinct plasma Aβ42/Aβ40 profiles. We hypothesized that associations between plasma biomarkers and memory will enable the identification of individuals at risk of ADRD in the community.

2. Methods

2.1 Study Setting and Participants

The Monongahela-Youghiogheny Health Aging Team (MYHAT) is an ongoing population-based study cohort drawn from a Rust Belt region of southwestern Pennsylvania, USA. These are formerly vibrant steel-manufacturing towns that never recovered from the economic blows of the steel industry's collapse in the 1970s. MYHAT participants are followed annually for the development of mild cognitive impairment (MCI) and dementia. Participants were selected by age-stratified random sampling from the publicly available voter registration lists over two time periods: 2006–2008 and 2016–2019. Inclusion criteria at study entry included: 1) 65+ years old, 2) living in a designated town, 3) not residing in long-term care settings, 4) having sufficient hearing and vision to complete neuropsychological testing, and 5) having decisional capacity. Recruitment procedures in 2016 for the new cohort were identical to those of 2006, except that participants were limited to the 65–74 age group, so as to replenish the cohort with participants ten years younger than the youngest members of the initial cohort. At initial recruitment, n=2,036 and n=708 participants provided written informed consent in the first and second recruitment phases respectively. All participants were briefly assessed using the Mini Mental State Examination (MMSE)[19]. Since the study investigates the epidemiology of MCI, we screened out those who already showed substantial cognitive impairment by scoring <21/30 on the age-education-corrected MMSE[20]. The full assessment was then administered to n=1,982 and n=703 participants in the original and second recruitment cohorts respectively. All study procedures were approved by the University of Pittsburgh Institutional Review Board and all participants provided written informed consent.

2.2 Study Assessments

Detailed assessment interviews included:

Demographics: age, sex, education (less than eighth grade or eighth to eleventh grade [<HS]; graduated from high school or GED [=HS]; graduated from college, 4-year college program or graduate school [>HS]), and self-identified race/ethnicity (White; Black or African American, more than one race, unknown or not reported [non-White]).

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Clinical Dementia Rating: At each annual assessment, certified research interviewers rated participants based on independence in cognitively-driven everyday activities using the Clinical Dementia Rating (CDR)®[21]. CDR was categorized into three groups: 0=normal, 0.5=MCI, ≥1=dementia.

Neuropsychological tests: At each visit, participants were administered a battery of neuropsychological tests tapping five cognitive domains: memory, attention, language, executive function, and visuospatial ability. Here, we focus on the memory domain. A composite score for the memory domain was generated by first standardizing each individual test score (Fuld Object Memory Evaluation[22], Wechsler Memory Scale-Revised Logical Memory and Visual Reproduction[23], and modified 12-item Face Name Associative Memory Exam[24]) and then calculating the mean of all the standardized scores in the memory domain.

Blood collection.: For those recruited in the initial 2006–2008 cohort, blood samples were collected during the annual assessments in 2014 or later. For the new cohort participants, blood was collected at visits in 2016 or later. Venous blood was collected in the morning following overnight fasting into purple-top ethylenediaminetetraacetic acid tubes. Samples were incubated at room temperature for 30–45 minutes, then centrifuged at 2,000 g for 10 min, 4°C. The plasma was collected into polypropylene tubes and stored at −80°C until use. Less than 10% of the participants (n=87) self-reported that they did not follow the overnight fasting procedure. However, we have shown that this does not significantly affect plasma biomarkers[25], and we confirmed that the results for these 87 participants did not differ from the rest of the cohort.

Apolipoprotein E (*APOE*) genotyping: Genotyping was performed using blood or saliva specimens. Genotypes for the *APOE*/rs429358 (*APOE*4*) and *APOE*/rs7412 (*APOE*2*) single-nucleotide polymorphisms (SNPs) were determined using TaqMan genotyping assays. Because of the strong linkage disequilibrium between the two SNPs, this is also treated as a three-allele *APOE* polymorphism: *APOE*2, APOE*3,* and *APOE*4*, resulting in six genotypes (2/2, 2/3, 2/4, 3/3, 3/4, 4/4)[25]. Individuals with any ε4 allele (2/4,3/4,4/4) were classified as *APOE*4* carriers and those without an ε4 allele as non-carriers.

2.3 Plasma biomarker measurements

Plasma biomarker concentrations were measured in singlicates using Single molecule array (SIMOA) methods on an HD-X instrument (Quanterix, Billerica, MA, USA) at the Department of Psychiatry, University of Pittsburgh School of Medicine, USA. All frozen samples underwent a single thawing cycle. Plasma p-tau181 was measured with the p-tau181 V2 Advantage (#103714) while NfL, GFAP, Aβ42 and Aβ40 concentrations were measured with the Neurology 4-Plex E (#103670) commercial assays from Quanterix (Billerica, MA, USA). For each assay, two or three quality control samples of different concentrations were analyzed in duplicates both at the start and the end of each technical run to estimate reproducibility. The pooled quality control data showed that the within-run (p-tau181=4.6–8.9%, NfL=10.9–17.7%, GFAP=6.6–13.2%, Aβ42=5.0–12.7% and Aβ40=5.8–

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13.5%) and between-run (p-tau181=10.8–13.5%, NfL=17.1–19.5%, GFAP=12.4–23.2%, Aβ42=9.0–17.0% and Aβ40=12.1–17.1%) variations in signal were mostly <20%.

2.5 Statistical analyses

Statistical analyses were performed using R 4.1.3[26]. We first compared the demographics for participants whose plsma samples were available versus not available. We then examined descriptive statistics of biomarkers and baseline demographics overall and by CDR group or a binary CDR variable (CDR=0 normal vs CDR≥0.5, MCI/dementia). Medians and interquartile ranges were calculated for each continuous variable; frequencies and percentages were calculated for categorical variables. We performed Kruskal-Wallis tests for continuous variables and Fisher's exact tests for categorical variables to compare biomarker distributions among CDR groups.

We further performed Kruskal-Wallis tests to compare biomarker distributions among age (65–74, 75–84, and 85+ years old) and education (<HS: less than high school, =HS: high school, >HS: higher than high school) groups, and Wilcoxon rank-sum tests to compare biomarker distributions between sex and *APOE*4* carrier and non-carrier groups.

To classify individuals into homogeneous plasma Aβ42/40 and Aβ42 groups, we applied an unsupervised clustering method: K-medoids[27], a more robust version of K-means which minimizes the distance between points labeled as being in the same cluster. All biomarker values were given as natural log-transformed; the distance matrix was calculated using the Euclidean distance, and the number of clusters was fixed to 2 since we aimed to find the threshold of two one-directional groups. We tested the difference in charasteristics (demographics and biomarkers) among groups. For each cluster, we further examined the correlations between pairs of biomarkers and between the memory composite and biomarkers using Spearman's correlation[28]. We further tested the above-mentioned associations by stratifying according to age, sex, education, or *APOE*4* allele individually using Spearman's correlation. To find the directions and magnitude of the above-mentioned associations overall or by strata, we fit robust linear regression, alternatives to least squares regressions when data are contaminated with outliers or influential observations, for each pair of variables of which we examined correlations. The associations of the memory composite score and biomarkers were similarly examined by CDR group.

3. Results

3.1 Participant and plasma biomarker characteristics

Among total 2685 participants in MYHAT study, plasma samples were available from 920 participants. The distributions of age at study entrance, sex, race and education were all significantly different between people who gave plasma samples versus those who did not. The demographic characteristics of the participants who consented to blood collection and were thus included in this study agreed with previous reports[29, 30]; younger, more females, more self-identified White, and more highly educated (Supp Table. 1 and Supp Fig. 1). After further excluding 19 participants missing one or more biomarker values, we had data from 901 participants. Biomarker levels below the assays' quantification limits

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were assigned the manufacturer-provided lower limits of detection values (Aβ40=0.384, Aβ42=0.136, NfL=0.09, GFAP=0.441, and p-tau181=0.028). In this way, we reassigned 2 values for Aβ40, 5 values for Aβ42 and 2 values for p-tau181.

No biomarker presented a normal distribution without transformation (Supp Fig. 2). While Aβ42/40 ratio, GFAP, NfL, and p-tau181 were unimodal and right-skewed, Aβ40 and Aβ42 showed bimodal distributions. After natural log transformations (Supp Fig. 3), Aβ42/40 was still slightly right-skewed; p-tau181, NfL, and GFAP were normally distributed. We removed n=54 outliers (red rectangles; Supp Fig. 3) which were out of the outer fence (Q1 – 3*IQR, Q3 + 3*IQR) and separated from the bulk values in the histograms; Aβ42/40 (n=2), p-tau181 (n=31), NfL and GFAP (identical n=21), leaving n=847 participants for the final analyses.

The characteristics of the 847 MYHAT participants by CDR groups are presented in Table 1. There were 125(14.8%) participants with MCI and 10(1.2%) with dementia, with the rest being cognitively normal individuals (~85%). The median(Q1, Q3) age of the cohort was 74.0(69.0, 83.0) years; 465 (54.9%) were aged 65–74 years, 216 (25.5%) aged 75–84 years, and the rest above 85 years. 306(36.1%) were male; 809(95.5%) were White; and 179 (21.1%) were *APOE*4* carriers. Sex and race distributions were similar among the three CDR groups. The participants in the CDR≥0.5 group were significantly older, less educated, and had a higher proportion of *APOE*4* carriers than the CDR=0 group. The median Aβ42/40 for the CDR=0 was slightly higher versus the CDR≥0.5 group. Median Aβ40 was significantly lower in the CDR >=1 group. Plasma p-tau181, NfL, and GFAP were each higher in the CDR≥0.5 group (Table. 1).

3.2 Distributions of plasma biomarkers according to demographics

As shown in Supp Fig. 4A, Aβ40 and Aβ42 were each significantly higher and Aβ42/40 ratio significantly lower in the 65–74-year-olds compared with the older age groups, whereas the levels were comparable between the 75–84 and 85+ age groups. Conversely, plasma p-tau181, GFAP, and NfL were each higher in 75–84 and 85+ age groups versus the 65–74-year-olds. Aβ42/40, GFAP, and NfL were significantly higher, whereas p-tau181 was lower, in females versus males (Supp. Fig. 4B). When stratified according to education (Supp. Fig. 4C), there was no significant difference in Aβ42/40. However, NfL, GFAP, and p-tau181 were each lower in the =HS and >HS groups compared with the <HS group . After adjusting for age, there were no differences in the distributions of plasma biomarkers among education levels except that NfL was still significantly lower in the ≥HS education group compared to the <HS education group among people aged 65–74. *APOE*4* carriers had significantly lower Aβ42, Aβ42/40, and NfL values, but non-significantly higher GFAP and p-tau181 levels (Supp. Fig. 4D).

3.3 Clustering according to plasma Aβ42/40 ratio and p-tau181 identifies two separate modes that reveal an intermediate group

The clustering result for Aβ42/40 (Fig. 1A) identified a bimodal distribution for both Aβ42 and Aβ40 (Fig. 1E–F), in agreement with previously reported CSF Aβ results[31, 32]. The optimal Aβ42/40 threshold to differentiate the two clusters was −2, resulting in

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2 Aβ42/40 modes: *normal* (N=730) and *abnormal* (N=117). Plotting plasma Aβ42/40 ratio against p-tau181 also identified an *abnormal* and a *normal* group which showed pathophysiological AD and biomarker-negative profiles, respectively (since p-tau181 is specifically higher according to AD pathology[10, 33–36]), according to associations between the plasma biomarkers. The boundary of the new clusters became a diagonal line with a positive slope instead of a vertical line at the −2 threshold for Aβ42/40 ratio alone (Fig. 1B), suggesting that the clustering result of the Aβ42/40 ratio depended on p-tau181. Some participants with slight Aβ42/40 suprathreshold and subthreshold values might still be in the *normal* versus *abnormal* cluster respectively. This allowed the definition of a third, "*uncertain*", group (blue rectangle in Fig. 1C) to include these individuals. As a result, the participants were clustered into three groups based on the log-transformed Aβ42/40: *normal* (−4, −2.3), *uncertain* (−2.3, −1.7), and *abnormal* (−1.7, 1). The *uncertain* group was established as +/−0.3 units around the original −2 threshold. Table. 2 shows the characteristics of participants by those 3 groups. There were 40 participants in the *uncertain* group, 97 in the *abnormal* group, and 710 in the *normal* group. Participants in the *non-normal* groups were older and less educated and included more females when compared with those in the *normal* group. Fig. 1B–C illustrate that data points in the *uncertain* group were more spread out (similar to those in the *abnormal* group and contrary to the closely packed *normal* group), suggesting that individuals in the *uncertain* group were in an intermediate state. Fig. 1D–G show the histograms of Aβ42/40, Aβ40, Aβ42, and p-tau181 respectively color-filled according to the different group of Aβ42/40. Whilst the color-coded Aβ40 and Aβ42 distributions showed overlaps between groups, a clear separation of all three groups was observed when using the Aβ42/40.

3.4 Associations between plasma Aβ42/40 modes and other plasma biomarkers

After determining the distinct modes of plasma Aβ42/40, we tested associations between plasma biomarker values for each Aβ42/40 mode (Fig. 1C and Fig. 2). When considering the two modes, there were significant inverse associations between Aβ42/40 and each of p-tau181 (ρ= −0.11, P=0.002; Fig. 1B), NfL (ρ= −0.11, P=0.002) and GFAP (ρ= −0.17, P<0.001; Fig 2C–D) in the *normal* mode. When split into three groups, the significant negative correlations between Aβ42/Aβ40 and each of p-tau181 (normal: ρ= −0.12, P=0.001; abnormal: ρ = −0.21, P=0.042) and NfL (*normal*: ρ= −0.18, P<0.001; *abnormal*: ρ= −0.26, P=0.011) was strongest in the *abnormal* group whilst the association with GFAP (*normal*: ρ= −0.24, P<0.001; *abnormal*: ρ= −0.21, P=0.043) was similar in the *normal* versus *abnormal* groups. No significant associations between plasma Aβ42/Aβ40 and other biomarkers were recorded in the *uncertain* group, potentially because of its comparatively small size.

3.5 Associations between plasma biomarkers and memory composite score by plasma Aβ42/40 mode groups

Among the five cognitive domains (attention, executive, language, memory, and visuospatial), memory deficit defines "amnestic" MCI [37]. We therefore focused on the association between the memory composite score and plasma biomarkers. When grouped by Aβ42/40 groups (Fig. 3A–D), the memory composite score showed a stronger negative correlation with p-tau181 in the *abnormal* (ρ= −0.33, P<0.001) versus the *normal* mode

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(ρ= 0.09, P=0.042). For NfL, the inverse association was stronger in the uncertain versus *abnormal* group (*abnormal*: ρ= −0.23, P=0.026; *uncertain*: ρ= −0.40, P=0.017) but non-existent in the normal group. Association between memory and GFAP was limited to the *abnormal* group (ρ= −0.22, P=0.036). Similar results were obtained when considering the two modes only (without the *uncertain* group; Fig. 3E–H). There were no significant associations between the memory composite score and plasma Aβ42/40 after adjusting for age, sex, education, or *APOE*4* (Supp Fig. 5A). However, there was a significantly negative association between memory and p-tau181 (Supp Fig. 5B) in the Aβ42/40 *abnormal* group in females, non-*APOE*4* carriers, and people above high school education. The memory composite score of Aβ42/40-*abnormal* females, >HS educated people, and non- *APOE*4* carriers was inversely associated with NfL after the adjustments (Supp Fig. 5C). The negative association was also present among 85+-year-olds with *normal* Aβ42/40 profiles. When controlled for all covariates except age, >75-year-olds and with a *normal* Aβ42/40 profile showed inverse association between GFAP and memory; additionally, females or highly educated individuals within the abnormal mode showed associations between memory composite score and GFAP (Supp Fig. 5D).

3.6 Associations between plasma biomarkers and memory composite score in CDR groups by plasma Aβ42/40 mode

In CDR=0 individuals, plasma NfL and GFAP were each positively associated with the memory composite score in the *normal* Aβ42/40 group (Supp Fig. 6A). Plasma p-tau181 showed an inverse association with memory composite score in the *abnormal* Aβ42/40 group (Supp Fig. 5B). Among CDR≥0.5 individuals with *normal* Aβ42/40 profiles, p-tau181 was negatively correlated with memory composite score (Supp Fig. 6A–B).

4.0 Discussion

We have described the profiles of ADRD plasma biomarkers in the population-based MYHAT cohort. Plasma p-tau181, GFAP, and NfL levels were higher in older individuals. The bimodal Aβ42/40 (or Aβ42) profiles (in agreement with CSF results[31, 32]) separated the population into two modes; participants with an *abnormal* Aβ42/40 profile had stronger associations with plasma NfL, p-tau181 and GFAP compared to those with a *normal* profile. Furthermore, plasma p-tau181 was associated with composite memory score, pointing to its utility to identify potentially at-risk individuals with or without cognitive impairment. Additionally, combining plasma Aβ42/40 with p-tau181 allowed us to apply the model more specifically to detect probable biomarker evidence of AD. Associations of NfL and GFAP with the memory composite in the normal Aβ42/40 modes/groups provide some validation to the notion that a negative Aβ42/40 profile might also be a potential marker for non-AD neurodegenerative diseases, and thus might help identify older adults at risk of those conditions. Future work, expanding our investigations into additional cognitive domains might help further clarify this issue.

The age-, cognition- and *APOE*4* carriership-associated higher levels in plasma p-tau181, GFAP, and NfL and lower levels in Aβ42/40 are in line with recent CSF/neuroimaging studies[10, 12, 38]. Notably, females showed higher GFAP and NfL levels, also corroborating

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recent findings[39]. Lower plasma p-tau181 in females has also been reported[40]. While Aβ42/40 ratio was not affected by education, NfL, GFAP, and p-tau181 were lower in the more-educated groups. Education can increase brain reserve, i.e., resistance to brain pathology, and could be reflected in plasma biomarker abnormalities[41]. Since the older participants were from a less-educated generation, this could be simply age-driven. However, after adjusting for age, the difference among different education levels mostly disappeared, suggesting that age has minimal effects on the results.

One of the most promising potentials and achievable goals of plasma biomarkers is population screening to identify at-risk older adults for further clinical and/or research evaluations [7, 42]. However, despite dozens of reports that plasma biomarkers associate strongly with CSF/neuroimaging biomarkers[9, 10, 43] and can even predict neuropathologic diagnosis[33, 44], studies examining their utility at the population level are lacking. Plasma Aβ42/40 were bimodally distributed, just as in CSF and Aβ-PET[7], enabling identification of two Aβ42/40-dependent modes. Associations with memory composite and other biomarkers suggested that the *abnormal* mode was enriched for individuals at risk for AD irrespective of cognitive status whilst the *normal* mode included CDR=0 and CDR≥0.5 participants potentially affected by non-AD neurodegenerative diseases. Clustering jointly with Aβ42/40 and p-tau181 allowed for validation, given the specificity of p-tau181 to AD[10, 33–36]. The strength of associations of the modes/groups with NfL, GFAP and p-tau181 were higher according to Aβ42/40 *abnormality*. Similarly, Giudici *et al.* showed that plasma Aβ42/40 classifies older adults into *low*, *intermediate* and *high risk* groups of Aβ-PET abnormalities[45]. Our clustering efficiency and interdependence with p-tau181 were stronger with Aβ42/40 versus Aβ42 alone in line with CSF results[46, 47].

In plasma, Aβ40, Aβ42 and the Aβ42/40 ratio have inverse relationships with their equivalent levels in the brain as measured with Aβ-PET [7, 8, 48]. This suggests that individuals with higher levels of these plasma Aβ peptide levels have lower brain amyloidosis whilst those with lower plasma Aβ levels have higher brain amyloidosis [7, 8, 48]. In agreement with previous reports[49, 50], our findings suggest that cognitively impaired groups demonstrated lower plasma Aβ42/40 suggesting higher likelihood of brain amyloidosis. Furthermore, plasma NfL, GFAP and p-tau181 showed stronger associations with memory performance in the *abnormal* compared with the *normal* and *intermediate* mode/groups, also indicating that participants with an *abnormal* Aβ42/40 have higher odds for neurodegeneration, glial actication and AD pathophysiology. These results persisted in the CDR=0 participants, suggesting that plasma biomarker changes occur before cognitive symptoms appear[38], and thus demonstrating potential effectiveness to identify at-risk community-dwelling individuals without cognitive concerns.

In AD, being the leading cause of cognitive impairment[51], one of the earliest pathophysiological changes is a decrease of Aβ42/40 ratio levels in plasma[8, 48, 50, 52]. Other biological changes including abnormal tau phosphorylation, neurodegeneration and inflammatory alterations tend to be evident after Aβ42/40 reduction[38, 53, 54]. This explains why Aβ42/40 ratio clustering identified groups of individuals with different plasma biomarker and cognitive profile associations. The results corroborate what has been shown for CSF Aβ42/40 and Aβ PET[54, 55]. The results indicate that plasma Aβ42/40 has a high

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screening value in identifying both symptomatic and asymptomatic individuals at significant risk of AD, to be eventually confirmed by CSF/neuroimaging tests. Confirmatory tests on this enriched sub-population would significantly reduce the number of individuals and the associated time and costs compared to assessing the entire cohort with CSF/neuroimaging tests.

An additional strength is that we used Aβ42 and Aβ40 immunoassay methods from Quanterix, that are more widely available, cost-effective and easier-to-implement alternatives to the immunoprecipitation-mass spectrometry (IP-MS) methods only accessible in a few research and clinical laboratories. Although previous studies suggested that immunoassay Aβ methods perform less favorably than IP-MS Aβ assays, we used improved immunoassays with superior antibody performances[38]. The Aβ42 and Aβ40 assays were shown in a recent study to perform superior to plasma p-tau181, GFAP and NfL to identify abnormal brain Aβ status in a cohort of cognitively normal older adults[38]. Furthermore, our clustering method is independent of age and *APOE*4* genotype, making it more practical compared with other approaches like the Amyloid Probability Score developed using a IP-MS plasma Aβ method[45].

The clustering method may also be useful for the differential prognosis of AD from other neurodegenerative diseases; older adults with *abnormal* Aβ42/40 or Aβ42 profiles and high p-tau181 should be at higher odds for AD whilst the *normal* profiles may include participants with non-AD neurodegenerative diseases in addition to unaffected individuals. Associations between plasma NfL and GFAP in the *normal* Aβ42/40 mode/group will be important to evaluate neurodegeneration and glial activation independent of AD.

The key novelty of this report is the data-driven approach that separates participants into plasma Aβ42/40- or Aβ42-dependent clusters with distinct p-tau181, NfL and GFAP association profiles according to the cohort characteristics. This approach can be applied to other cohorts to accelerate threshold generation for plasma biomarkers, just as it was done for CSF biomarkers. Our findings should be replicated in other population-based studies, particularly those with greater racial/ethnic diversity.

The study's main strength is the use of a well-characterized community based cohort. The three-group approach described also has an advantage of identifying individuals with incipient disease compared with the two-group approach that only classifies individuals as positive and negative. Moreover, as the study sample was randomly sampled from the voter registration list, it is not subject to the selection bias typical of studies conducted in clinical settings. However, since the sample is of largely European ancestry, our findings should be replicated in population samples with greater racial and ethnic diversity[56].

In conclusion, we have shown, in the population-based MYHAT cohort, that plasma biomarkers associate with cognitive impairment, *APOE*4* carriership and older age. Additionally, we demonstrate a clustering model to identify individuals at risk of AD pathophysiology. Once replicated in other population-based cohorts, these results will be important to screen for biomarker evidence of AD in older adults with or without cognitive

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concerns. This strategy will help enrich for individuals with biological evidence of disease for inclusion in intervention trials, early detection and longitudinal monitoring campaigns.

Supplementary Material

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Funding

The authors thank all MYHAT study personnel for their efforts and all MYHAT participants for their time, data, and specimens. M.G is supported by the NIH (R37 AG023651). PCLF is supported by Alzheimer's Association (AARFD-22-923814). BB is supported by CAPES [88887.336490/2019-00] and Alzheimer's Association (AARFD-22-974627). BS is supported by the National Institute of Aging (R01 AG023651). TAP is supported by the National Institute of Aging (R01AG075336, R01AG073267) and the Alzheimer's Association (AACSF-20-648075). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Union's Horizon Europe research and innovation programme under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, and #ADSF-21-831377-C), the Bluefield Project, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), and the UK Dementia Research Institute at UCL (UKDRI-1003). TKK was funded by the Swedish Research Council (Vetenskapsrådet #2021-03244), the Alzheimer's Association (#AARF-21-850325), the Swedish Alzheimer Foundation (Alzheimerfonden), the Aina (Ann) Wallströms and Mary-Ann Sjöbloms stiftelsen, and the Emil och Wera Cornells stiftelsen.

Abbreviations:

Aβ	Amyloid-β
ADRD	Alzheimer's disease and related disorders
AD	Alzheimer's disease
APOE	Apolipoprotein E
CDR	Clinical Dementia Rating
CSF	Cerebrospinal fluid
GFAP	Glial Fibrillary Acidic Protein
HS	High School
IP-MS	Immuno-Precipitation Mass Spectrometry
MCI	Mild Cognitive Impairment
MMSE	Mini Mental State Examination
MYHAT	Monongahela-Youghiogheny Health Aging Team
NfL	Neurofilament Light
PET	Positron Emission Tomography
P-tau181	Phosphorylated-tau181

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SIMOA	Single Molecule Array

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Systematic review:

We searched PubMed for plasma biomarkers of Alzheimer's disease and related disorders (ADRDs). Dozens of studies have shown that: plasma Aβ42/40, GFAP and p-tau181 associate with brain Aβ pathology; p-tau181 correlates with brain tau pathology; and NfL is a strong indicator of neurodegeneration. Consequently, we sought to apply these tools to identify community-dwelling older adults with at-risk biomarker and clinical profiles.

Interpretation:

Bimodal distribution of plasma Aβ42/40 ratio allowed classification of n=847 population-based participants into three main groups. Plasma NfL, GFAP and p-tau181 correlated strongest with Aβ42/40 ratio and memory composite in the *abnormal* group. Furthermore, significant associations were observed in the *normal* and *uncertain* Aβ42/40 groups, suggesting sensitivity to identify individuals with emerging ADRD pathophysiology.

Future directions:

Future studies are needed to validate these results in other population-based cohorts and examine the capacity of biomarkers to identify people with incipient ADRD for clinical monitoring and/or inclusion in therapeutic trials.

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Highlights

- Population-based plasma biomarker studies are lacking, particularly in cohorts without CSF or neuroimaging data;
- In the MYHAT study (n=847), plasma biomarkers associated with worse memory and Clinical Dementia Rating, *APOE*ε4, and greater age;
- Plasma Aβ42/40 ratio levels allowed clustering participants into *abnormal, uncertain* and *normal* groups;
- Plasma Aβ42/40 correlated differently with NfL, GFAP, p-tau181, memory composite and Clinical Dementia Rating in each group;
- Plasma biomarkers will enable relatively affordable and non-invasive community screening for evidence of ADRD pathophysiology

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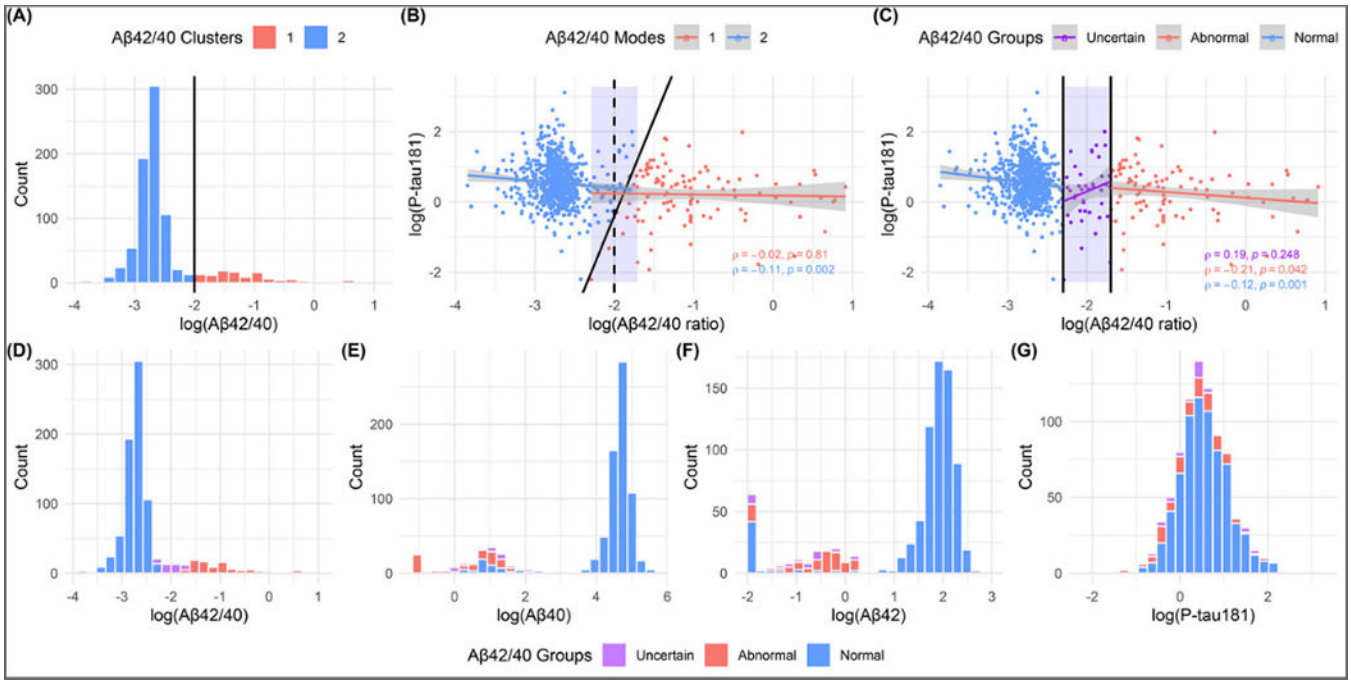

Figure 1. Top: Clustering results and modes/groups for plasma Aβ42/40 ratio.

The figure **A** shows the distribution for Aβ42/40 ratio filled by its K-medoids clustering result; the cutoff point is −2. Figure **B** is the scatterplot for plasma Aβ42/40 ratio and p-tau181 colored by their K-medoids clustering result. The black dash line is the cutoff point for Aβ42/40 ratio mapped to 2-dimension. The scatterplot in figure **C** shows the association between Aβ42/40 ratio and p-tau181 colored by three groups. The groups (*Normal*: < −2.3, *Uncertain*: [−2.3, −1.7], and *Abnormal*: > −1.7) are defined based on the plasma Aβ42/40 ratio values. The black solid lines are the boundaries of modes or groups. The blue rectangles are the uncertain group. **Bottom: Distributions of biomarkers**. The histograms of plasma (**D**) Aβ42/40 ratio, (**E**) Aβ40, (**F**) Aβ42, and (**G**) p-tau181 filled by Aβ42/40 ratio groups. Aβ = amyloid β, p-tau181 = tau phosphorylated at threonine 181, GFAP = glial fibrillary acidic protein, NfL = neurofilament light.

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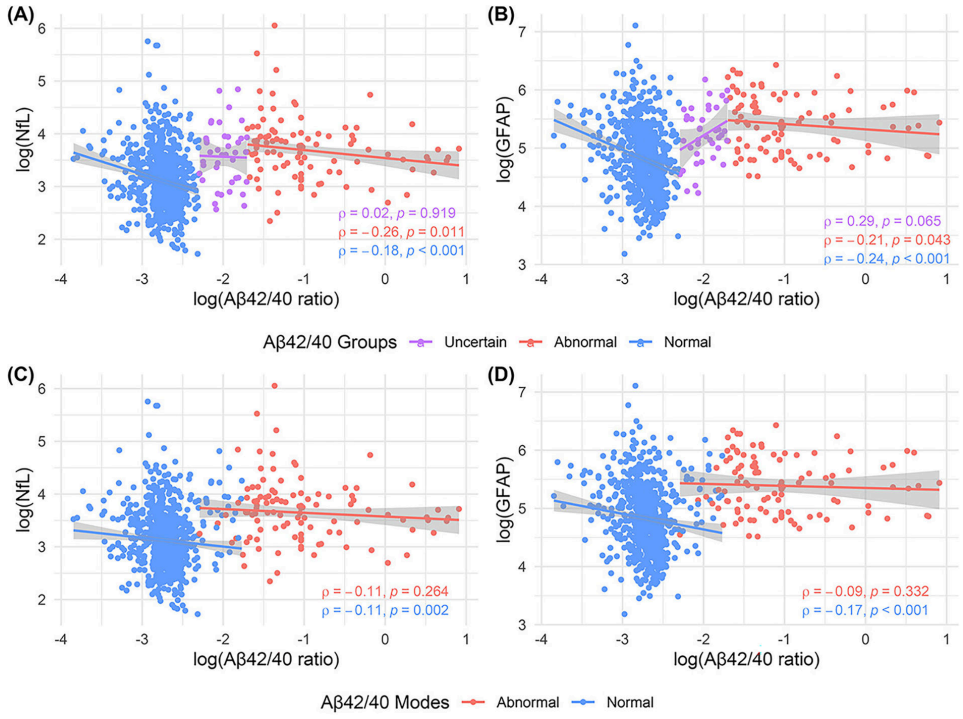

Figure 2. Associations between plasma biomarkers by Aβ42/40 ratio modes and groups.

The upper panel shows the association between plasma Aβ42/40 ratio and (**A**) NfL and (**B**) GFAP by Aβ42/40 ratio groups (*Normal*, *Uncertain*, and *Abnormal*; defined using pre-defined cutoffs). The lower panel shows the association between plasma Aβ42/40 ratio and (**C**) NfL and (**D**) GFAP by Aβ42/40 ratio modes (*Normal*, and *Abnormal*; defined based on previous clustering results). All statistical associations were tested using Spearman's correlation. Each individual point is colored based on plasma Aβ42/40 ratio modes or groups. All statistical tests were two-sided with no adjustment for multiple comparisons. Shaded areas represent 95% confidence intervals of the robust linear regression lines. Aβ = amyloid β, p-tau181 = tau phosphorylated at threonine 181, GFAP = glial fibrillary acidic protein, NfL = neurofilament light. The side-by-side presentation of plasma Aβ42/40 ratio associations with the other plasma biomarkers in the three- versus two-group clusters allows for a demonstration of how consideration of the intermediate zone affects these associations.

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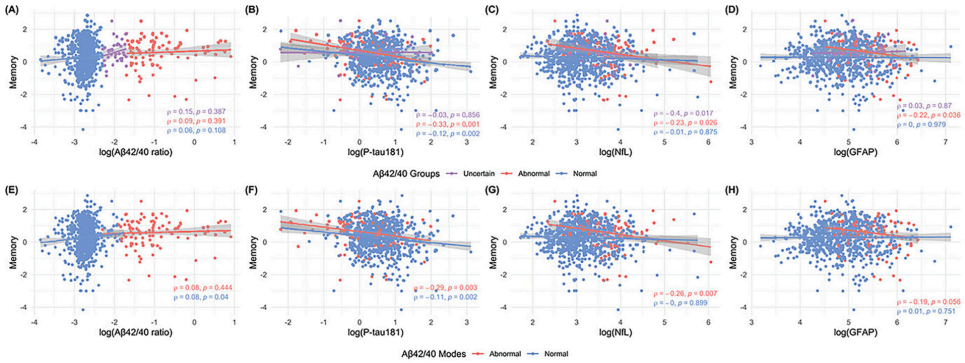

Figure 3. Associations between memory composite score and plasma biomarkers by Aβ42/40 ratio modes and groups.

The upper panel shows the association between memory composite score with plasma (**A**) Aβ42/40 ratio, (**B**) p-tau181, (**C**) NfL, and (**D**) GFAP by Aβ42/40 ratio groups (*Normal*, *Uncertain*, and *Abnormal*; defined using pre-defined cutoffs). The lower panel shows the association between memory composite score with plasma (**E**) Aβ42/40 ratio, (**F**) p-tau181, (**G**) NfL, and (**H**) GFAP by Aβ42/40 ratio modes (*Normal*, and *Abnormal*; defined based on previous clustering results). All figures are annotated with Spearman's rho rank correlations and corresponding unadjusted two-sided p-values. Points are colored by plasma Aβ42/40 groups or modes. The regression lines are fitted by robust linear regression and shaded areas represent the 95% confidence intervals. Aβ = amyloid β, p-tau181 = tau phosphorylated at threonine 181, GFAP = glial fibrillary acidic protein, NfL = neurofilament light. The side-by-side presentation of plasma Aβ42/40 ratio associations with composite meomory scores in the three- versus two-group clusters enabled evaluation of how the intermediate zone alters the relationships.

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Table 1.

Participant characteristics, median (Q1, Q3) or N (%), by CDR scores.

	Normal (N=712) (CDR=0)	MCI (N=125) (CDR=0.5)	Dementia (N=10) (CDR≥1)	Total (N=847)	p-value*
Age, years					*< 0.001*
Median	73.00	80.00	89.50	74.00	
Q1, Q3	69.00, 81.00	70.00, 87.00	88.00, 91.00	69.00, 83.00	
Age group, N (%)					< 0.001
65–74-year-olds	414 (58.1%)	50 (40.0%)	1 (10.0%)	465 (54.9%)	
75–84-year-olds	186 (26.1%)	30 (24.0%)	0 (0.0%)	216 (25.5%)	
85+-year-olds	112 (15.7%)	45 (36.0%)	9 (90.0%)	166 (19.6%)	
Sex, N (%)					*0.829*
Male	260 (36.5%)	43 (34.4%)	3 (30.0%)	306 (36.1%)	
Female	452 (63.5%)	82 (65.6%)	7 (70.0%)	541 (63.9%)	
Race, N (%)					*0.272*
White	683 (95.9%)	116 (92.8%)	10 (100.0%)	809 (95.5%)	
Non-White	29 (4.1%)	9 (7.2%)	0 (0.0%)	38 (4.5%)	
Education, N (%)					*< 0.001*
< High School	26 (3.7%)	16 (12.8%)	2 (20.0%)	44 (5.2%)	
= High School	250 (35.1%)	51 (40.8%)	4 (40.0%)	305 (36.0%)	
> High School	436 (61.2%)	58 (46.4%)	4 (40.0%)	498 (58.8%)	
***APOE*4, N (%)**					*< 0.001*
Non-carriers	577 (81.0%)	88 (70.4%)	3 (30.0%)	668 (78.9%)	
Carriers	135 (19.0%)	37 (29.6%)	7 (70.0%)	179 (21.1%)	
Aβ40, pg/mL					*0.069*†
Median	4.60	4.63	2.89	4.60	
Q1, Q3	3.93, 4.77	4.37, 4.86	0.75, 4.89	4.04, 4.78	
Aβ42, pg/mL					*0.323*†
Median	1.86	1.86	1.22	1.86	
Q1, Q3	1.18, 2.07	1.55, 2.06	−0.77, 2.02	1.28, 2.07	
Aβ42/Aβ40 ratio					*0.023*†
Median	−2.66	−2.72	−2.69	−2.67	
Q1, Q3	−2.80, −2.51	−2.87, −2.59	−2.85, −2.16	−2.81, −2.52	
p-tau181, pg/mL					*< 0.001*†
Median	0.45	0.77	0.86	0.49	
Q1, Q3	0.11, 0.83	0.40, 1.11	0.45, 1.14	0.14, 0.89	
NfL, pg/mL					*< 0.001*†
Median	3.11	3.41	3.96	3.15	
Q1, Q3	2.81, 3.51	2.94, 3.73	3.49, 4.24	2.83, 3.57	
GFAP, pg/mL					*< 0.001*†
Median	4.85	5.07	5.51	4.88	

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	Normal (N=712) (CDR=0)	**MCI (N=125) (CDR=0.5)**	**Dementia (N=10) (CDR≥1)**	**Total (N=847)**	**p-value***
Q1, Q3	4.48, 5.23	4.68, 5.48	5.21, 5.66	4.50, 5.29	

*: Kruskal-Wallis tests were used for continuous variables with non-parametric distributions, whereas Fisher Exact tests were used for categorical variables.

†: Plasma biomarkers natural log transformed to better approximate normality and variance homogeneity. MCI: mild cognitively impaired. <HS : less than eighth grade or eighth to eleventh grade; =HS: graduated from high school or GED; >HS: graduated from college, 4-year college program or graduate school. Non-White: White; Black or African American, more than one race, unknown or not reported. Aβ: amyloid beta, p-tau181: phosphorylated-tau 181, GFAP: glial fibrillary acidic protein, NfL: neurofilament light chain.

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Table 2.

Participant characteristics, median (Q1, Q3) or N (%), by 3 Aβ42/Aβ40 ratio groups.

	Normal (N=710) (−4, −2.3)	Uncertain (N=40) (−2.3, −1.7)	Abnormal (N=97) (−1.7, 1)	p-value*
Age, years				< 0.001
Median	73.00	82.00	83.00	
Q1, Q3	69.00, 80.00	77.00, 87.00	77.00, 87.00	
Age group, N (%)				< 0.001
65–74-year-olds	445 (62.7%)	8 (20.0%)	12 (12.4%)	
75–84-year-olds	155 (21.8%)	16 (40.0%)	45 (46.4%)	
85+-year-olds	110 (15.5%)	16 (40.0%)	40 (41.2%)	
Sex, N (%)				0.036
Male	269 (37.9%)	13 (32.5%)	24 (24.7%)	
Female	441 (62.1%)	27 (67.5%)	73 (75.3%)	
Race, N (%)				0.363
White	675 (95.1%)	39 (97.5%)	95 (97.9%)	
Non-White	35 (4.9%)	1 (2.5%)	2 (2.1%)	
Education, N (%)				0.044
< High School	34 (4.8%)	2 (5.0%)	8 (8.2%)	
= High School	243 (34.2%)	17 (42.5%)	45 (46.4%)	
> High School	433 (61.0%)	21 (52.5%)	44 (45.4%)	
***APOE* *4, N (%)**				0.333
Non-carriers	554 (78.0%)	32 (80.0%)	82 (84.5%)	
Carriers	156 (22.0%)	8 (20.0%)	15 (15.5%)	
Aβ40, pg/mL				< 0.001†
Median	4.66	1.24	0.64	
Q1, Q3	4.46, 4.83	0.55, 1.95	−0.96, 1.11	
Aβ42, pg/mL				< 0.001†
Median	1.93	−0.66	−0.43	
Q1, Q3	1.71, 2.11	−1.42, −0.11	−0.97, −0.12	
Aβ42/Aβ40 ratio				< 0.001†
Median	−2.71	−2.00	−1.06	
Q1, Q3	−2.83, −2.61	−2.11, −1.83	−1.42, −0.63	
p-tau181, pg/mL				< 0.001†
Median	0.52	0.36	0.34	
Q1, Q3	0.19, 0.91	−0.27, 0.73	−0.22, 0.74	
NfL, pg/mL				< 0.001†
Median	4.80	5.21	5.40	
Q1, Q3	4.44, 5.16	4.98, 5.58	4.94, 5.75	
GFAP, pg/mL				< 0.001†
Median	3.06	3.62	3.70	

	Normal (N=710) (−4, −2.3)	Uncertain (N=40) (−2.3, −1.7)	Abnormal (N=97) (−1.7, 1)	p-value*
Q1, Q3	2.78, 3.44	3.15, 3.92	3.35, 3.97	

*: Kruskal-Wallis tests were used for continuous variables with non-parametric distributions, whereas Chi-square tests were used for categorical variables.

†: Plasma biomarkers natural log transformed to better approximate normality and variance homogeneity. Aβ: amyloid beta, p-tau181: phosphorylated-tau 181, GFAP: glial fibrillary acidic protein, NfL: neurofilament light chain.

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