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# Plasma phosphorylated tau 217 and amyloid- $\beta$ 42/40 for amyloid risk in subgroups

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## Abstract

**Background** Alzheimer's disease (AD) is characterized by the accumulation of amyloid- $\beta$  (A $\beta$ ) pathology. Recently, plasma biomarkers, particularly p-tau217, have emerged as promising tools for early diagnosis and risk stratification. In this retrospective study, we evaluated the diagnostic performance of p-tau217 combined with other plasma biomarkers in distinguishing A $\beta$  Positron emission tomography (PET) positivity in cognitively unimpaired (CU) and cognitively impaired (CI) individuals across diverse clinical subgroups.

**Methods** We analyzed 2,497 participants from the Korea-Registries to Overcome dementia and Accelerate Dementia (K-ROAD) cohort, including 636 CU and 1,971 CI individuals. Plasma p-tau217 was measured using both Single MOlecule Array (SIMOA) and Meso Scale Discovery (MSD) assays, alongside A $\beta$ 42/40, Glial fibrillary acidic protein (GFAP), and Neurofilament light chain (NfL). We assessed the diagnostic performance of biomarker combinations for A $\beta$  PET positivity through the area under the receiver operating characteristic curve (AUC), Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), and performed subgroup analyses based on age, sex, body mass index (BMI), and Apolipoprotein E (APOE)  $\epsilon$ 4 status. To assess applicability, we stratified the cohort by recruitment site into a development set (Samsung Medical Center,  $n = 1,545$ ) and a validation set (other centers,  $n = 952$ ).

**Results** In CU individuals from the development cohort, the combination of p-tau217 and A $\beta$ 42/40 significantly improved diagnostic accuracy (AUC: ALZpath 0.937 vs. 0.905, MSD 0.901 vs. 0.861;  $p < 0.05$ , DeLong test; 95% CIs) and model fit (AIC/BIC,  $p < 0.001$ ) compared to p-tau217 alone. In contrast, in CI individuals, the combination provided only modest improvements in model fit without significantly enhancing AUC. GFAP and NfL did not contribute

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significantly to amyloid detection in either group. These findings were successfully validated in an independent cohort from other centers. Subgroup analyses in CU individuals showed the greatest improvements in older adults, females, and APOE4 non-carriers, regardless of obesity status. In CI individuals, the combination had no significant impact on AUC except in males, where a small but significant increase was observed ( $p=0.002$ ).

**Conclusion** Combining p-tau217 with A $\beta$ 42/40 enhances amyloid detection in CU individuals, improving both diagnostic accuracy and model fit, whereas its impact in CI individuals is limited. These results highlight the potential of plasma biomarker combinations for refining early AD diagnostics and individualized risk assessment.

**Keywords** Alzheimer's disease, Plasma biomarkers, p-tau217, A $\beta$ 42/40, Diagnostic accuracy

## Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of  $\beta$ -amyloid (A $\beta$ ) plaques and tau neurofibrillary tangles [1–3]. The pathological cascade of AD is thought to begin with A $\beta$  deposition, which can be detected even in cognitively unimpaired (CU) individuals before clinical symptoms emerge [4, 5]. Currently, A $\beta$  positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) biomarkers are the gold standard for detecting A $\beta$  pathology, enabling early diagnosis and risk stratification [6–8]. However, their widespread clinical use remains limited due to high costs, invasiveness, and logistical constraints.

In recent years, plasma biomarkers have emerged as promising, minimally invasive alternatives for detecting AD pathology [9–12]. Among these, plasma phosphorylated tau (p-tau) 217 is highly specific for AD and strongly correlates with both A $\beta$  and tau pathology [13–16]. Additionally, the A $\beta$ 42/40 ratio reflects soluble amyloid species and serves as an early marker of amyloid accumulation [17–19]. While GFAP and NfL are considered non-specific biomarkers [20, 21], they play critical roles in AD pathophysiology—GFAP reflecting astrocytic activation and NfL serving as a marker of neurodegeneration [22–26]. Despite the growing use of plasma biomarkers in AD diagnostics, there is limited research on which biomarkers should be combined with p-tau217 to maximize diagnostic accuracy [27–31]. Furthermore, the optimal biomarker combination may differ depending on cognitive status. In CU individuals, where amyloid accumulation often precedes significant tau pathology, early markers like A $\beta$ 42/40 may provide complementary information to p-tau217 [29, 32]. However, it remains unclear whether and how the utility of such combinations may differ across cognitive stages and clinical subgroups. Clarifying the optimal biomarker combinations for each context is essential to improving plasma-based AD diagnostics. In this regard, additional biomarkers such as NfL and GFAP—which reflect neurodegeneration and astrocytic activation, respectively—may capture broader pathological changes and contribute to model performance in certain subgroups, though their specificity is limited.

In contrast, in cognitively impaired (CI) individuals—where both A $\beta$  and tau pathology are more prevalent—it is possible that biomarkers reflecting neurodegeneration, such as NfL, or neuroinflammation, such as GFAP, could improve diagnostic performance by capturing broader disease processes beyond A $\beta$  pathology [33, 34]. However, the specific benefits of these additional biomarkers in different cognitive stages remain unclear, highlighting the need for further research on context-specific plasma biomarker combinations.

Unlike AD pathologies detected in the brain, plasma biomarkers are subject to greater biological variability due to peripheral influences, which can impact their diagnostic accuracy and interpretation. Among these influences, demographic and clinical factors such as age, sex, body mass index (BMI), and Apolipoprotein E (APOE)  $\epsilon$ 4 carrier status play a significant role in modulating plasma biomarker levels [35–37]. Age-related changes in neurodegeneration, sex-based hormonal and metabolic differences, BMI-associated variations in A $\beta$  metabolism, and the impact of APOE  $\epsilon$ 4 on A $\beta$  processing all contribute to differences in biomarker concentrations across individuals. Given these variations, subgroup analyses based on these factors are essential to assess whether the diagnostic performance of combined plasma biomarkers differs across populations. Identifying these differences could help refine biomarker-based screening strategies and improve individualized risk assessment for AD.

In the present study, we evaluated the diagnostic performance and model fit of a plasma biomarker combination in a large cohort of CU and CI individuals, with p-tau217 measured using both the Single MOlecule Array (SIMOA) and Meso Scale Discovery (MSD) assays. We further validated this biomarker model in an independent subset from other centers to assess its generalizability. Additionally, we aimed to determine whether the effectiveness of this combined biomarker model varies by stratifying participants based on demographic and clinical factors, including age, sex, BMI, and APOE  $\epsilon$ 4 status.

## Materials and methods

### Study population

Participants were consecutively recruited from the Korea-Registries to Overcome dementia and Accelerate Dementia (K-ROAD) project, a multicenter nationwide initiative involving 25 tertiary-care hospitals across South Korea between 2016 and 2024, with Samsung Medical Center (SMC) serving as the core institution. Although individuals with non-AD etiologies such as frontotemporal dementia (FTD) and subcortical vascular cognitive impairment (SVCI) were also enrolled in the broader K-ROAD cohort, these participants were excluded from the current analysis to focus on the Alzheimer's disease continuum. Accordingly, the current study included only individuals along the Alzheimer's disease continuum—cognitively unimpaired (CU), mild cognitive impairment (MCI), and dementia of the Alzheimer's type (DAT). These participating tertiary hospitals not only recruited patients from memory disorder clinics, similar to ADNI and the Amsterdam Dementia Cohort, but also operated government-commissioned dementia prevention centers established by local municipalities to support community-based dementia screening and care. This hybrid recruitment strategy reflects both clinical and population-based referrals, enhancing the representativeness of the cohort [38].

We analyzed a total of 2,497 individuals who underwent both plasma biomarkers testing and A $\beta$  PET imaging. For this retrospective study, we included participants from two groups: CU ( $n=613$ ) and CI ( $n=1,884$ ), with the CI group consisting of individuals diagnosed with MCI ( $n=1,344$ ) or DAT ( $n=540$ ). To examine the broader applicability of the model within the cohort, we stratified the data by recruitment site: SMC ( $n=1,545$ ) and other participating centers ( $n=952$ ). Inclusion criteria for CU, MCI, and AD dementia were based on established diagnostic frameworks. MCI due to AD was defined according to the NIA-AA criteria [39], and cognitive status was assessed using the Korean version of the Mini-Mental State Examination-2: Standard Version (K-MMSE-2:SV) [40] and the Seoul Neuropsychological Screening Battery (SNSB) [41]. Full inclusion and exclusion criteria are detailed in the Supplementary Methods.

### Brain magnetic resonance imaging acquisition

All participants underwent brain magnetic resonance imaging (MRI) at their respective centers, following a standardized imaging protocol. This protocol included 3-dimensional (3D) T1 turbo field echo sequences and fluid attenuated inversion recovery (FLAIR) imaging, using a 3.0-T MRI scanner. T1-weighted images were obtained with an isotropic voxel size of 1 mm<sup>3</sup> on all MRI machines. All images were reviewed at Samsung Medical Center. The median time between A $\beta$  PET imaging and

plasma collection was 4 days, with an interquartile range of 0–69 days.

### A $\beta$ PET imaging acquisition and quantification using RdcCL methods

All participants underwent A $\beta$  PET imaging with [<sup>18</sup>F]Florbetaben or [<sup>18</sup>F]Flutemetamol, according to the manufacturer's imaging guidelines. We then quantified A $\beta$  uptake using the global MRI-based regional direct comparison Centiloid (rdcCL) method [42]. Global amyloid PET positivity was defined using a regional data-driven Centiloid (rdcCL) threshold of 20, which has been widely used in both research and clinical trial settings to define abnormal amyloid burden, including in the AHEAD study (NCT04468659). This cutoff offers an objective and standardized criterion that facilitates cross-site comparability [43, 44]. All imaging analyses were conducted at the Alzheimer's Disease Convergence Research Center at SMC. The detailed protocol for PET imaging, quantification and obtaining A $\beta$  PET cutoff points is described in the Supplementary Methods.

### Plasma collection and processing

The detailed plasma collection and processing methods are described in Supplementary Methods. Plasma A $\beta$ 40, A $\beta$ 42, GFAP, and NfL concentrations were measured using the commercial Neurology 4-Plex E kit (Quanterix, PN 103670). The p-tau217 concentration was measured using two different immunoassay platforms. First, measurements were performed using the commercial ALZpath Simoa<sup>®</sup> p-tau217 v2 assay (ALZpath) kit (Quanterix, PN 104371) at the University of Gothenburg, Sweden. Second, additional p-tau217 measurements were obtained using a customized assay on the Meso Scale Discovery (MSD) platform (Rockville, Maryland), conducted by Lilly Research Laboratories. The median interval between plasma collection and A $\beta$  PET imaging was 0 days (IQR 0–21 days). Laboratory personnel conducting the biomarker assays were blinded to the participants' clinical information and A $\beta$  PET results.

### Stratification by potential influencing factors affecting plasma p-tau217 level

We first evaluated the diagnostic performance of p-tau217 alone and p-tau217 combined with another biomarker for predicting A $\beta$  PET positivity in participants classified into the CI and CU groups. In each group, the optimal biomarker combination was selected as the best model. The performance of the p-tau217 alone model and the best combination model was then validated in subgroups stratified by age, sex, BMI, and APOE  $\epsilon$ 4 carrier status to examine how model performance varies across different demographic subgroups.

Statistical analysis

All eligible participants with complete plasma biomarker and amyloid PET data ( $N=2,497$ ) were included in the overall dataset. No formal sample size calculation was performed, as the study utilized all available data from the K-ROAD cohort. Demographic and clinical characteristics were summarized as mean (standard deviation) or median (interquartile range) for continuous variables, and as numbers (percentages) for categorical variables. Model development was conducted using data from participants recruited at SMC. Logistic regression models were used to predict A $\beta$  PET positivity based on plasma biomarker data. Model performance was evaluated using area under the receiver operating characteristic curve (AUC), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and likelihood ratio tests (LRTs). AUC comparisons between the p-tau217-only model and combination models were conducted using DeLong's test. Multivariate logistic regression models used to compute the amyloid probability (AP), and optimal cut-offs were derived based on the Youden Index. The resulting AP model was applied to an independent subset from other participating centers, and performance metrics, including AUC, sensitivity, specificity, PPV, and NPV, were calculated for both cohorts.

Additionally, pre-specified subgroup analyses were conducted in the entire K-ROAD cohort to evaluate model performance across demographic and clinical factors, including age, sex, APOE  $\epsilon 4$  status, and BMI.

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). The data analysts were blinded to participants' clinical diagnoses and A $\beta$  PET status during model development and evaluation.

Results

Baseline characteristics of the study population

The baseline characteristics of the study population are summarized in Table 1.

All participants included in this analysis ( $N=2,497$ ) had complete data for both plasma biomarkers and amyloid PET Centiloid values, as shown in the participant flow diagram (Supplementary Fig. 1). Among CI participants ( $N=1,884$ ) and CU participants ( $N=613$ ), the mean age was 71.8 and 70.1 years, respectively, with 20.9% and 25.0% under 65 years. Females comprised 62.9% of the CI group and 64.3% of the CU group. The mean BMI was 23.5 in the CI group and 24.0 in the CU group, with obesity ( $BMI \geq 25 \text{ kg/m}^2$ ) observed in 27.4% and 34.1%, respectively. APOE  $\epsilon 4$  carriers accounted for 44.2% of the CI group and 25.1% of the CU group, while A $\beta$  positivity was found in 63.9% and 26.6%, respectively. Table 1 also presents a stratified comparison of participant characteristics between Samsung Medical Center ( $n=1,545$ ) and other centers ( $n=952$ ). Overall, participants from other centers tended to be slightly older and had lower education levels, particularly in the CI group. APOE  $\epsilon 4$  carriage and A $\beta$  PET positivity were more common in SMC participants across both CI and CU groups, whereas the proportion of younger and female participants was slightly higher at other centers in the CU group.

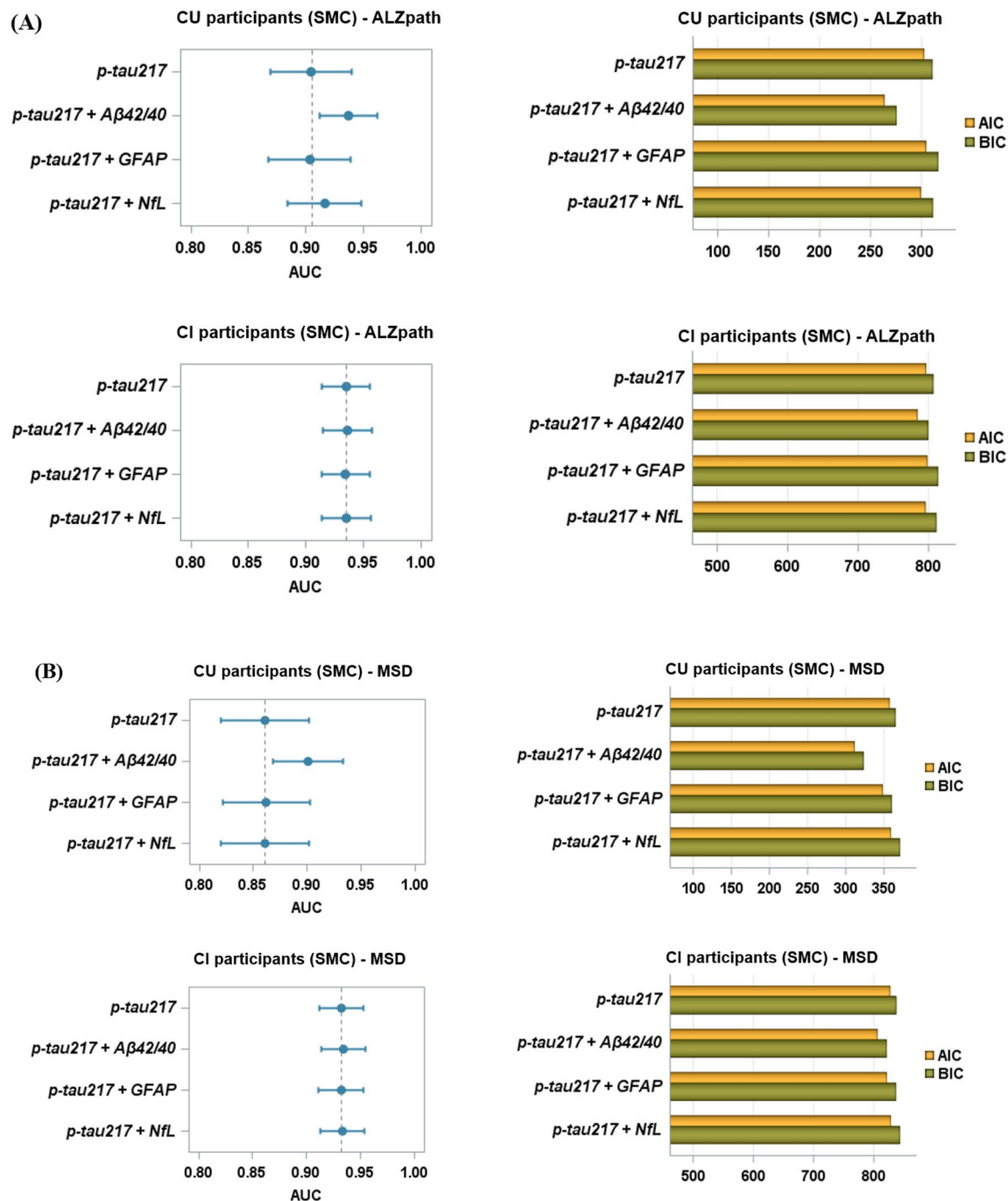
Development of plasma biomarker models in the SMC cohort

In CU individuals, the combination of p-tau217 and A $\beta 42/40$  ratio significantly improved diagnostic accuracy compared to p-tau217 alone (ALZpath: 0.937 vs. 0.905,  $p=0.001$ , DeLong test; MSD: 0.901 vs. 0.861,  $p=0.004$ , DeLong test) (Fig. 1 and Supplementary Table S1). Additionally, the combination model showed better model fit, with lower AIC/BIC values (ALZpath: 262.7/274.6 vs.

Table 1 Baseline characteristics of participants

| Characteristics                              | Total (N=2,497)             |                | Samsung Medical Center (N=1,545) |                | Other centers (N=952)      |                |
|----------------------------------------------|-----------------------------|----------------|----------------------------------|----------------|----------------------------|----------------|
|                                              | CI (N=1,884)                | CU (N=613)     | CI (N=391)                       | CU (N=1,154)   | CI (N=730)                 | CU (N=222)     |
| Age, mean (SD), years                        | 71.8 (8.7)                  | 70.1 (8.3)     | 71.2 (8.7)                       | 71.4 (7.3)     | 72.8 (8.7)                 | 67.7 (9.4)     |
| Age < 65, n (%)                              | 393 (20.9%)                 | 153 (25.0%)    | 273 (23.66%)                     | 69 (17.65%)    | 120 (16.44%)               | 84 (37.84%)    |
| Female, n (%)                                | 1,185 (62.9%)               | 394 (64.3%)    | 708 (61.35%)                     | 242 (61.89%)   | 477 (65.34%)               | 152 (68.47%)   |
| Years of education, mean (SD)                | 10.6 (4.8)                  | 11.4 (4.7)     | 11.3 (4.8)                       | 11.9 (4.7)     | 9.4 (4.6)                  | 10.6 (4.6)     |
| BMI, mean (SD), kg/m <sup>2</sup>            | 23.5 (3.2)                  | 24.0 (2.9)     | 23.4 (3.1)                       | 24.0 (3.0)     | 23.6 (3.3)                 | 24.1 (2.8)     |
| Obese (BMI $\geq 25 \text{ kg/m}^2$ ), n (%) | 517 (27.4%)                 | 209 (34.1%)    | 313 (27.19%)                     | 123 (31.87%)   | 204 (28.1%)                | 86 (38.91%)    |
| $\epsilon 4$ carriers, n (%)                 | 832 (44.2%)                 | 154 (25.1%)    | 559 (48.44%)                     | 113 (28.9%)    | 273 (37.4%)                | 41 (18.47%)    |
| Cognitive stage (MCI / DAT), n (%)           | 1,344 (71.3%) / 540 (28.7%) | Not Applicable | 702 (60.83%) / 452 (39.17%)      | Not Applicable | 642 (87.95%) / 88 (12.05%) | Not Applicable |
| A $\beta$ positivity (rdcCL > 20), n (%)     | 1,203 (63.9%)               | 163 (26.6%)    | 829 (71.84%)                     | 123 (31.46%)   | 374 (51.23%)               | 40 (18.02%)    |

Abbreviations: CU, Cognitively Unimpaired; CI, Cognitively Impaired; SD, Standard deviation; n, Number of individuals; BMI, Body mass index; APOE, Apolipoprotein E; MCI, Mild cognitive impairment; DAT, Dementia of Alzheimer's type; rdcCL, regional direct comparison Centiloid



**Fig. 1** Diagnostic performance of combined plasma biomarkers measured by ALZpath (A) and MSD (B) in CU and CI individuals from the development cohort of the K-ROAD study, recruited at SMC. The figures present the diagnostic performance of plasma p-tau217 alone and in combination with Aβ42/40, GFAP, or NfL in CU and CI individuals using the ALZpath assay (A) and the MSD assay (B). In both panels, the left graphs depict the AUC values for each biomarker combination, with the dashed gray line representing the AUC of p-tau217 alone. The right graphs present model fit comparisons using AIC and BIC, where lower values indicate better model fit. The error bar indicates 95% confidence interval. Abbreviations: SMC, Samsung Medical Center; MSD, Meso Scale Discovery; CU, cognitively unimpaired; CI, cognitively impaired; GFAP, Glial fibrillary acidic protein; NfL, Neurofilament light chain; AUC, area under the curve; AIC, Akaike information criterion; BIC, Bayesian information criterion

301.7/309.6,  $p < 0.001$ ; MSD: 310.3/322.1 vs. 356.1/364,  $p < 0.001$ ). In contrast, adding GFAP or NfL to p-tau217 did not yield further improvements. In CI individuals, the combination did not significantly improve AUC

compared to p-tau217 alone (ALZpath: 0.936 vs. 0.935,  $p = 0.353$ , DeLong test; MSD: 0.934 vs. 0.932,  $p = 0.567$ ). However, model fit was still improved, as reflected by lower AIC/BIC values (ALZpath: 783.1/798.3 vs.

795.3/805.4,  $p < 0.001$ ; MSD: 804.5/819.6 vs. 825.8/835.9,  $p < 0.001$ ).

The optimal AP cutoffs were 0.255 (ALZpath) and 0.250 (MSD) for the p-tau217 + Aβ42/40 model, and 0.229 (ALZpath) and 0.322 (MSD) for the p-tau217-only model in CU individuals. In CI individuals, the cutoffs were 0.580 (ALZpath) and 0.592 (MSD) for the combination model, and 0.528 (ALZpath) and 0.536 (MSD) for the p-tau217-only model.

Validation of plasma biomarker models in the independent cohort

To assess generalizability, we applied the derived AP cut-offs to an independent subset from other centers (Table 2). In CU individuals, combining biomarkers yielded higher AUCs than p-tau217 alone (ALZpath: 0.954 vs. 0.935; MSD: 0.951 vs. 0.907) and comparable or improved sensitivity (ALZpath: 90.0%; MSD: 85.0%), specificity (ALZpath: 90.1%; MSD: 88.2%), and NPV (ALZpath: 97.6%; MSD: 96.3%). In CI individuals, AUCs remained similar (ALZpath: 0.930 vs. 0.928; MSD: 0.927 vs. 0.923), with modest gains in model fit, sensitivity, and specificity.

Plasma p-tau217 and Aβ42/40 combination across subgroups

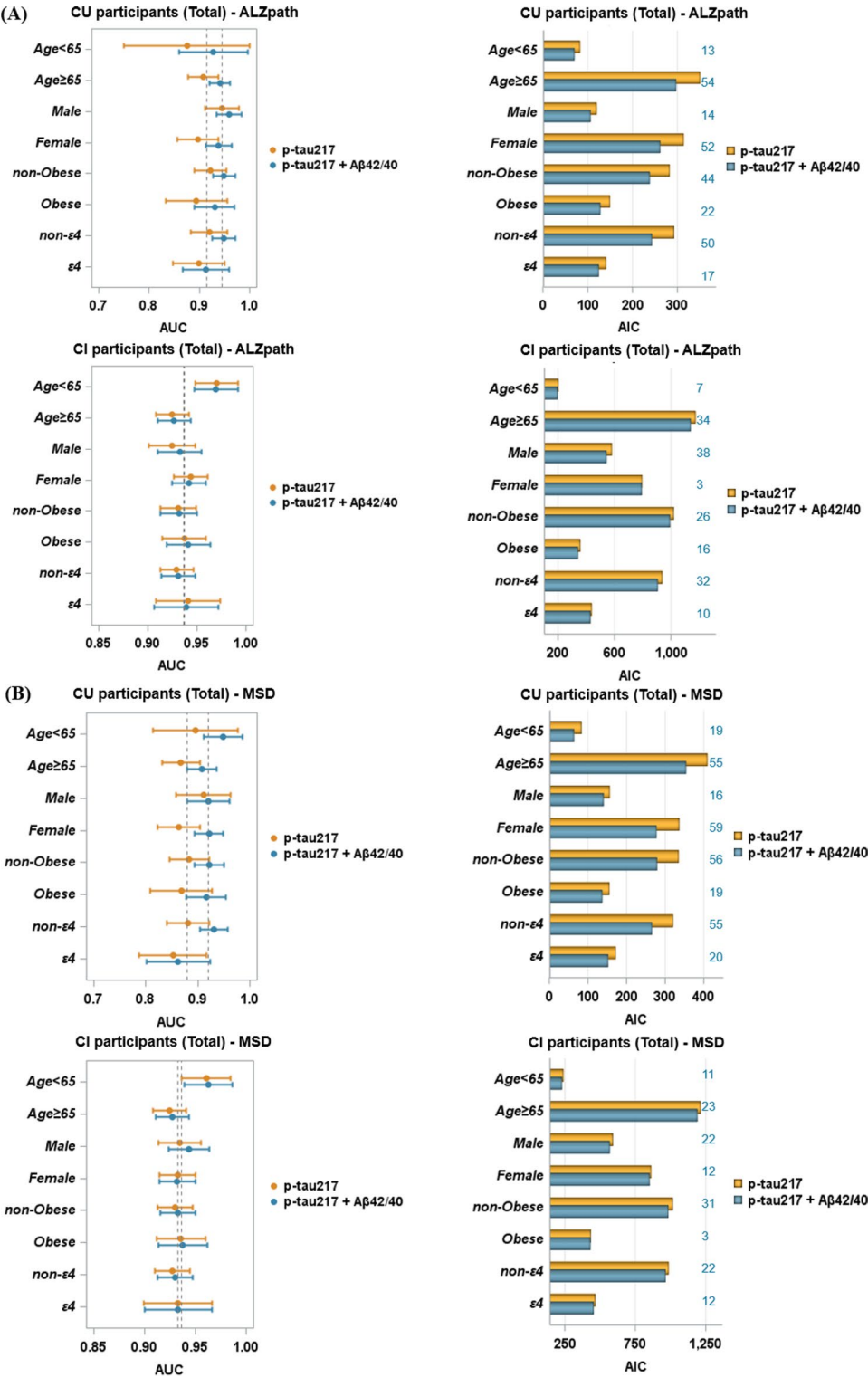
Given the consistent findings across both the development and validation cohorts, and to ensure sufficient statistical power in subgroup analyses, we combined the two datasets. We then evaluated the diagnostic performance of the p-tau217 + Aβ42/40 ratio across subgroups defined by age, sex, BMI, and APOE ε4 status, as this model outperformed other p-tau217-based combinations (Fig. 2 and Supplementary Table S2)

In CU individuals, the combination significantly improved AUC in older adults ( $\geq 65$  years, from 0.908 to 0.940,  $p = 0.001$ ), females (from 0.897 to 0.938,  $p < 0.001$ ), non-obese individuals (from 0.922 to 0.948,  $p = 0.006$ ), obese individuals (from 0.894 to 0.930,  $p = 0.033$ ), and non-APOE ε4 carriers (from 0.919 to 0.949,  $p = 0.0086$ ) in the ALZpath assay. Similar improvements were observed with the MSD assay, where the results were comparable to those obtained with the ALZpath assay. Across all CU subgroups, the combination model also demonstrated better fit, as evidenced by lower AIC values. In CI individuals, AUC differences between subgroups were minimal, except in males, where the combination significantly improved AUC in the ALZpath assay (0.925 to 0.932,  $p = 0.002$ ) and the MSD assay (0.935 to 0.944,  $p = 0.002$ ). However, AIC values were consistently lower across all

Table 2 Validation performance of the amyloid probability score in other centers

| Development cohort (SMC)          |                    | AUC   | AP    | Sensitivity | Specificity | PPV   | NPV   |
|-----------------------------------|--------------------|-------|-------|-------------|-------------|-------|-------|
| ALZpath                           |                    |       |       |             |             |       |       |
| CU                                | p-tau217           | 0.905 | 0.229 | 85.4%       | 84.0%       | 70.9% | 92.6% |
|                                   | p-tau217 + Aβ42/40 | 0.937 | 0.255 | 88.6%       | 85.8%       | 74.2% | 94.3% |
| CI                                | p-tau217           | 0.935 | 0.528 | 92.9%       | 88.0%       | 95.2% | 82.9% |
|                                   | p-tau217 + Aβ42/40 | 0.936 | 0.58  | 91.8%       | 89.5%       | 95.7% | 81.1% |
| MSD                               |                    |       |       |             |             |       |       |
| CU                                | p-tau217           | 0.861 | 0.322 | 75.6%       | 85.6%       | 70.3% | 88.6% |
|                                   | p-tau217 + Aβ42/40 | 0.901 | 0.25  | 90.8%       | 78.0%       | 65.1% | 94.9% |
| CI                                | p-tau217           | 0.932 | 0.536 | 93.3%       | 86.9%       | 94.8% | 83.5% |
|                                   | p-tau217 + Aβ42/40 | 0.934 | 0.592 | 92.0%       | 90.9%       | 96.3% | 81.5% |
| Validation cohort (Other centers) |                    | AUC   |       | Sensitivity | Specificity | PPV   | NPV   |
| ALZpath                           |                    |       |       |             |             |       |       |
| CU                                | p-tau217           | 0.935 |       | 90.0%       | 87.9%       | 62.1% | 97.6% |
|                                   | p-tau217 + Aβ42/40 | 0.954 |       | 90.0%       | 90.1%       | 66.7% | 97.6% |
| CI                                | p-tau217           | 0.928 |       | 87.4%       | 87.4%       | 87.9% | 86.9% |
|                                   | p-tau217 + Aβ42/40 | 0.930 |       | 84.5%       | 90.2%       | 90.0% | 84.7% |
| MSD                               |                    |       |       |             |             |       |       |
| CU                                | p-tau217           | 0.907 |       | 75.0%       | 92.7%       | 69.8% | 94.3% |
|                                   | p-tau217 + Aβ42/40 | 0.951 |       | 85.0%       | 88.2%       | 61.8% | 96.3% |
| CI                                | p-tau217           | 0.923 |       | 87.5%       | 88.1%       | 88.6% | 86.9% |
|                                   | p-tau217 + Aβ42/40 | 0.927 |       | 84.0%       | 91.0%       | 90.8% | 84.3% |

Abbreviations: AP, amyloid probability; MSD, Meso Scale Discovery; CU, Cognitively Unimpaired; CI, Cognitively Impaired; AUC, Area under curve; PPV, positive predictive value; NPV, Negative predictive value



**Fig. 2** Diagnostic performance of the plasma p-tau217 and Aβ42/40 combination across subgroups using ALZpath (A) and Meso Scale Discovery (B) assays. The figures present the diagnostic performance of the p-tau217 + Aβ42/40 combination across subgroups stratified by age, sex, body mass index, and APOE ε4 status, using the ALZpath assay (A) and the MSD assay (B). The left panels display the AUC values for each subgroup, comparing p-tau217 alone and the combination model. The dashed gray line represents the AUC of p-tau217 alone and the p-tau217 + Aβ42/40 model in the total participant cohort. The right panels illustrate model fit using AIC and BIC, where lower values indicate better model fit. The error bar indicates 95% confidence interval. Abbreviations: MSD, Meso Scale Discovery; CU, cognitively unimpaired; CI, cognitively impaired; AUC, area under the curve; AIC, Akaike information criterion; BIC, Bayesian information criterion

CI subgroups, suggesting modest model fit improvements despite the lack of significant AUC changes

## Discussion

We assessed the diagnostic performance and model fit of plasma biomarkers in CU and CI individuals using SIMOA and MSD assays, with model development at SMC and validation in an independent cohort. Our major findings were as follows. First, in CU individuals, the combined plasma p-tau217 and A $\beta$ 42/40 model significantly improved diagnostic accuracy and model fit compared to p-tau217 alone, whereas in CI, it provided only modest model fit improvements without enhancing AUC. These findings were consistent across both the SIMOA and MSD assays, and the amyloid probability score was validated in an independent cohort, supporting its robustness and cross-site applicability. In contrast, adding GFAP or NfL did not improve diagnostic performance or model fit in either group. Finally, within CU subgroups stratified by age, sex, APOE4 status, and obesity, the combined model enhanced diagnostic performance in older individuals, females, and APOE4 non-carriers. These findings were observed in both obese and non-obese participants and were consistent across both the SIMOA and MSD assays. Taken together, these findings suggest that the combined plasma p-tau217 and A $\beta$ 42/40 model improves predictive performance in specific CU subgroups, while offering only modest benefits in CI individuals. These results underscore the clinical potential of integrated plasma biomarker strategies for enhancing early diagnosis and individualized risk assessment of AD.

Our primary finding was that in CU individuals, the combined plasma p-tau217 and A $\beta$ 42/40 model significantly improved diagnostic accuracy and model fit compared to p-tau217 alone, whereas in CI individuals it provided only modest improvements in model fit without enhancing AUC. This difference in effectiveness likely stems from the distinct pathological profiles of these groups. Specifically, CI individuals exhibit a greater enrichment of tau pathology than CU individuals. Although p-tau217 is associated with both A $\beta$  and tau, its relationship in CU individuals is predominantly with amyloid, whereas in CI individuals—where tau pathology is more enriched—it is more closely linked to tau uptake. Consequently, in CI individuals, p-tau217 alone is highly accurate in identifying amyloid-positive cases, rendering the added value of A $\beta$ 42/40 minimal. In contrast, in CU individuals the sensitivity of p-tau217 for detecting early A $\beta$  accumulation may be limited; since A $\beta$ 42/40 reflects soluble A $\beta$  species that decline prior to A $\beta$  PET positivity, incorporating it into the model likely enhances early A $\beta$  detection.

We identified the optimal AP cutoffs were higher in CI than CU individuals, which aligns with our prior findings showing that p-tau217 levels are influenced predominantly by amyloid pathology in CU, and by both amyloid and tau pathology in CI [45]. This highlights the potential need for subgroup-specific thresholds in clinical applications.

Indeed, previous studies have indicated that the effectiveness of the p-tau217 and A $\beta$ 42/40 combination model is greater in CU individuals than in CI individuals. In studies focused solely on CI individuals, the APS2—derived from the %p-tau217 and A $\beta$ 42/40 combination—primarily enhanced model fit relative to the %p-tau217-only model, with minimal impact on AUC [28]. Conversely, in studies of CU individuals, models incorporating both %p-tau217 and A $\beta$ 42/40 demonstrated improved adjusted R<sup>2</sup> and overall model fit compared to models based exclusively on %p-tau217 [29, 31, 46]. Furthermore, in a study that separately analyzed CU and MCI groups, although direct comparisons were not performed, the combined model appeared to be more effective in the CU group [30]. Similar findings were also observed in another study employing the Fujirebio platform, reinforcing the notion that the added value of A $\beta$ 42/40 is particularly pronounced in the early, cognitively unimpaired stage [47]. These findings underscore the stage-dependent diagnostic value of plasma biomarkers in AD. Specifically, the enhanced performance of the combined model in CU individuals suggests its potential utility for early detection and patient stratification, which is crucial for timely intervention.

Conversely, GFAP and NfL, which primarily reflect neuroinflammation and neurodegeneration, did not substantially improve A $\beta$  detection in either group. While these markers have been associated with AD pathology and have shown potential for tracking disease progression [26, 48–50], their role in early A $\beta$  accumulation appears to be limited. In our study, although GFAP and NfL contributed modestly to model fit improvements, their impact was not as pronounced as that of A $\beta$ 42/40, particularly in CU individuals. This suggests that while GFAP and NfL may provide additional insights into neurodegenerative processes, they do not directly enhance the ability to detect A $\beta$  pathology in its earliest stages.

Our final major finding was that in CU individuals, the combined model significantly improved diagnostic performance in specific subgroups, particularly older individuals, females, and APOE4 non-carriers, regardless of obesity status, with consistent enhancements in AUC and model fit across both the SIMOA and MSD assays. These findings suggest that integrating p-tau217 and A $\beta$ 42/40 may enhance individualized risk assessment in CU. In contrast, while younger individuals, males, and APOE4 carriers showed a trend toward improved AUC

and model fit, these changes did not reach statistical significance. Interestingly, in CI individuals, the combination model did not significantly improve AUC across most subgroups, except in males, where a modest but significant increase was observed. The reason for this sex-specific effect remains unclear, highlighting the need for further investigation. These results underscore the potential of plasma biomarker combinations for refining individualized risk assessment and early detection strategies in AD. Further large-scale studies are needed to determine how demographic and genetic factors modulate their effectiveness.

Our study has several strengths. It utilized a large, well-characterized cohort with both plasma biomarker and A $\beta$  PET data, enabling development of diagnostic models and assessment of their generalizability across clinical and demographic strata. The cut-off-based amyloid probability score was further validated in an independent subset from other centers, supporting its applicability across different recruitment sites. However, several limitations should be acknowledged. First, although our overall sample size was large, some subgroups defined by age, sex, BMI, and APOE  $\epsilon$ 4 status, especially within the CU group, had relatively small sample sizes. Therefore, caution is warranted when interpreting subgroup-specific model improvements, and these findings should be validated in larger, independent cohorts. Second, although findings were consistent across both SIMOA and MSD assays, potential platform-specific variability in plasma biomarker measurements cannot be entirely excluded. Also, although logistic regression was used in this study, future work may explore clinically implementable alternatives such as decision trees or point-based scoring systems. Finally, the use of A $\beta$  PET positivity as the reference standard may not fully capture the complexity of AD pathology, particularly in early stages where tau accumulation and neurodegeneration also play critical roles. Despite these limitations, our study provides important evidence supporting the complementary role of p-tau217 and A $\beta$ 42/40 in detecting early A $\beta$  pathology, particularly in CU individuals. These findings underscore the potential of integrated plasma biomarker strategies to improve the accuracy and efficiency of AD diagnostics in preclinical stages.

In summary, our study demonstrates that combining p-tau217 with A $\beta$ 42/40 significantly enhances A $\beta$  detection in CU individuals, improving both diagnostic accuracy and model fit, whereas its impact in CI individuals is limited to modest model fit improvements. These findings highlight the complementary role of A $\beta$ 42/40 in capturing early A $\beta$  pathology and suggest that plasma biomarker combinations may offer a more sensitive approach for detecting preclinical AD.

This work may serve as a foundation for future research aimed at developing clinically practical approaches for biomarker-based risk assessment.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13195-025-01826-3>.

Supplementary Material 1

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### Author contributions

H.K. (Heekyoung), D.S., and S.S. conceptualized and designed the study and drafted the manuscript. S.Y., D.L.N., S.K., J.Y., M.C., E.L., J.K., and H.K. (Hee Jin) acquired the data. H.K. (Heekyoung), D.S., H.Y., J.L., and S.S. contributed to data curation and formal analysis. D.S. and S.S. provided critical review and editing of the manuscript. Funding was obtained by H.Z., K.B., F.G.O., N.J.A., H.J. and S.S. D.S. and S.S. supervised the study. All authors contributed to the final manuscript and were involved in the decision to submit it for publication.

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#### Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Declarations

##### Ethics approval and consent to participate

The institutional review board of Samsung Medical Center (No. 2021-02-135) approved this study. All participants provided informed consent to participate in the study, and the data were collected in accordance with the Declaration of Helsinki.

##### Consent for publication

Not applicable.

##### Competing interests

Henrik Zetterberg has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave. He has delivered lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche. He is also a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is part of the GU Ventures Incubator Program (outside the submitted work). Kaj Blennow has served as a consultant and advisory board member for Abbvie, AC Immune, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers. He has served on data monitoring committees for Julius Clinical and Novartis. He has also delivered lectures, produced educational materials, and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai, and Roche Diagnostics. Additionally, he is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is part of the GU Ventures Incubator Program, outside the work presented in this paper. All other authors declare no conflicts of interest. Theresa A. Day is an employee and minor stockholder of Eli Lilly and Company.

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