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Prediction of Alzheimer's disease diagnosis within 14 years through Aβ misfolding in blood plasma compared to *APOE4* status, and other risk factors

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Abstract

Introduction: Alzheimer's disease (AD) has a long prodromal stage and identifying high-risk individuals is critical. We aimed to investigate the ability of Aβ misfolding in blood plasma, *APOE4* status, and dementia risk factors to predict diagnosis of AD.

Methods: Within a community-based cohort, Aβ misfolding in plasma measured by immuno-infrared sensor and *APOE* genotype were determined at baseline in 770 participants followed over 14 years. Associations between Aβ misfolding, *APOE4*, and other predictors with clinical AD, vascular dementia, and mixed dementia diagnoses were assessed.

Results: Aβ misfolding was associated with a 23-fold increased odds of clinical AD diagnosis within 14 years. No association was observed with vascular dementia/mixed dementia diagnoses. *APOE4*-positive participants had a 2.4-fold increased odds of clinical AD diagnosis within 14 years.

Discussion: Aβ misfolding in blood plasma was a strong, specific risk prediction marker for clinical AD even many years before diagnosis in a community-based setting.

KEYWORDS
Alzheimer's disease, Amyloid beta (Aβ), Apolipoprotein E (*APOE*), Vascular dementia, Blood plasma, Risk stratification

1 | INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease, in which pathophysiological changes may precede clinical symptoms by many years.[1] AD is categorized through amyloid β (Aβ) plaques and tau tangles in the brain, which can be documented through postmortem examination or in vivo through brain biomarkers.[1] However, clinical AD diagnosis still only identifies the disease several years after its onset.[2,3] Given the lack of effective curative options after clinical AD diagnoses, and the long prodromal phase of AD, early identification of those at risk is imperative for the development of disease-modifying and preventative treatment strategies alike.[2,4]

AD risk models have previously been based on demographic, health, lifestyle, and genetic factors.[5] Apolipoprotein E ε4 allele (*APOE4*)

carrier status is the strongest genetic risk factor for AD and has been used and recommended for risk stratification in AD therapeutic development trials.[6–8] However, recently, it has been suggested that clinical trial inclusion should target AD pathological changes of AD.[9] Cerebrospinal fluid (CSF)-derived or positron emission tomography (PET) imaging biomarkers express physiological changes associated with disease pathology but are invasive or cost-prohibitive.[2,10–13] Emerging Aβ-targeted blood-based biomarkers or risk stratification strategies could present a promising alternative for early identification of AD pathological changes while being minimally invasive and cost-effective.[14,15]

One strategy to identify pathologic changes in blood plasma is through Aβ misfolding measurement. Aβ is known to undergo a structural change from monomeric, alpha-helical, or disordered conformations to β-sheet-enriched isoforms during disease progression and these β-sheet-enriched isoforms are the basis of plaque formation in the brain. It has been shown that Aβ misfolding not only is observable in the brain but also correlates to Aβ misfolding in CSF and plasma.[14,15] The focus of previous Aβ-targeted plasma studies has largely been on validation of a biomarker comparing patients with clinically manifest AD and healthy controls in a clinical setting.[15–17] The investigation in a community-based cohort since the introduction of more sophisticated Aβ measurement techniques with comparison to other dementia risk factors and dementia outcomes is lacking.

Therefore, the aim of this study was to assess the potential of Aβ misfolding as an early predictor of clinical AD risk and to compare it to *APOE4* status and other predictors of dementia risk in a community-based cohort followed over 14 years. A secondary aim of this study was to compare the ability of these risk indicators to predict clinical vascular dementia (VD) and mixed dementia (MD) diagnoses.

RESEARCH IN CONTEXT

1. Systematic review: In recent years, Aβ-targeted blood-based Alzheimer's disease (AD) biomarkers and risk markers have emerged as minimally invasive potential option for detecting AD pathology, yet the focus of previous studies has largely been on the validation of biomarkers comparing patients with clinically manifest AD and healthy controls.[1,2] Furthermore, the setting of previous research has also largely been restricted to a clinical setting warranting community- and population-based studies investigating Aβ-targeted blood-based AD risk stratification markers.
2. Interpretation: We assessed Aβ misfolding in blood plasma as a predictor of clinical AD diagnosis in a community-based cohort of older adults followed over 14 years. Participants with Aβ misfolding had a 23-fold increased odds of clinical AD diagnosis within 14 years.
3. Future directions: Epidemiological cohort studies play a key role in determining the predictive value of risk markers. Aβ misfolding in blood plasma could greatly enhance risk stratification in the therapeutic development setting and could benefit from confirmation in larger cohort studies and in a more diverse population to increase generalizability.

2 | MATERIALS AND METHODS

2.1 | Study design and population

The analyses are based on a nested case-control study embedded within the ESTHER study (Epidemiologische Studie zu Chancen der Verhütung Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung), a community-based prospective cohort study of older adults in Germany. Details of the cohort study and of the nested case-control approach have been given elsewhere.[18] Briefly, ESTHER consists of 9940 participants aged 50–75 years attending a general health examination offered starting at age 35 biannually in the German health care system who were recruited by their general practitioners (GPs) in a statewide study in Saarland, Germany, in 2000–2002. Participants completed standardized self-administered health questionnaires and provided blood samples, including heparin plasma samples, which were stored at −80°C. In addition, medical information was provided by the GPs and comprehensive follow-up with respect to incidence of major disease and mortality was conducted through follow-up questionnaires given to participants and their GPs 2, 5, 8, 11, 14, and 17 (ongoing) years after recruitment and by linkage with population registries and provision of death certificates of deceased participants by local health authorities, respectively. The ESTHER study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg and the Physicians' Board of Saarland. The nested case-control study within the ESTHER study consisted of 874 participants, 167 of whom were reported by their GPs to have been diagnosed with dementia during follow-up, and 707 controls without known dementia diagnosis randomly selected and frequency matched by baseline age, sex, and educational level. Briefly, GPs of participants that had dropped out due to health or death were contacted during the 14–year follow-up regarding dementia diagnoses. Of the surveys sent (n = 2455), 75% (n = 1843) were returned, of which 212 identified participants with dementia diagnoses including AD, VD, MD, frontotemporal dementia, or unspecified dementia. Clinical diagnoses of AD (n = 70), VD (n = 85), and MD (n = 40) were chosen for inclusion in the study, and for these groups, controls were randomly chosen among the rest of participants who had not dropped out of the ESTHER cohort and were frequency-matched by age, sex, and education (control to case ratio: 4:1). For this analysis, the three control groups were combined. Excluded participants included: 70 participants with hemolytic plasma samples, 19 cases where suspected dementia diagnosis could not be confirmed by further medical records, seven purported controls with a later identified dementia diagnosis, individuals without information on *APOE* genotype (6 AD, 9 VD, 2 MD, and 86 controls), and one additional participant who withdrew consent.[19]

2.2 | Biomarkers and clinical diagnoses

The blood plasma samples used in this study were collected at baseline and AD, VD, and MD diagnoses were collected from participants' GPs during the 14–year ESTHER follow-up as previously reported.[20] Briefly, GPs filled out a questionnaire regarding patient dementia diagnoses and provided all available medical records of neurologists, psychiatrists, memory, or other specialized providers. The current guidelines in Germany for AD diagnosis follow the National Institute on Aging and the Alzheimer's Association[21] or the International Working group (IWG)–2 criteria.[22,23]

Soluble Aβ peptides were extracted from frozen blood plasma at baseline, and alterations in Aβ peptide secondary structure were measured for each participant with an immuno-infrared sensor (WO 2015121339 A1), the details of which have been reported elsewhere.[19,24] Thus, this structure-based biomarker presents the misfolding state of Aβ in blood plasma.

The immuno-infrared sensor has been validated in detail, including generation and characterization of NHS-silane, antibody batch-to-batch variation, antibody performance with synthetic and standard reference CSF and blood plasma samples, matrix effects, lower and higher limits of quantification, assay selectivity, sample handling, and documentation of zero background signals after Aβ immunodepletion.[19,24–27] It should be noted that the immuno-infrared assay detects the Aβ secondary structure distribution as a relative measure and is thus robust and independent of concentration fluctuations and sample variation. Finally, all plasma sample analyses were performed in a blinded manner at the Department of Biophysics at Bochum University, Bochum, Germany.

APOE genotyping was performed using TaqMan SNP genotyping assays with genotypes analyzed in an endpoint allelic discrimination read using a PRISM 7000 Sequence detection system (Applied Biosystems, Foster City, CA) as previously described.[19] Additional comorbidities at baseline (i.e., diabetes, hypertension, cardiovascular events and depression) were self-reported and partially confirmed by physician reports.

2.3 | Statistical methods

Descriptive statistics were calculated to provide information on participant characteristics, while chi-square and t-tests were used to compare both AD cases, VD cases, MD cases, and the combined dementia cases occurring within 14 years of follow-up to controls (individuals without known dementia diagnosis). Multiple imputation (n = 12) for data missing at random was carried out following the Markov chain Monte Carlo method.[28] The number of imputations was determined by the percentage of participants with one or more missing values. The imputed data set was used for the calculation of the odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression based on each dementia risk factor including Aβ misfolding status, *APOE4* status, age, sex, education, physical activity, obesity, diabetes, hypertension, and history of cardiovascular events (myocardial infarction, stroke, death due to cardiovascular disease) and depression. Two models were used in logistic regression analyses that considered AD, VD, and any dementia (AD + VD + MD) cases occurring within 8 years, between 8 and 14 years, and within both time windows combined (i.e., the full 14 years of follow-up). The control group for all analyses included only participants without known dementia diagnosis (i.e., when predicting AD diagnosis, VD, MD cases were not included in the control group). Model 1 adjusted for age, sex, and education while model 2 additionally adjusted for all other covariates. In the logistic regression models, both Aβ misfolding status and *APOE4* status were considered as binary variables. In agreement with a previously validated spectral threshold,[19] participants with a cutoff of $<1642\ cm^{-1}$, the point at which the maximum position of the amide I absorbance band indicates Aβ misfolding, were considered to have Aβ misfolding present and participants with $\geq$1 *APOE* ε4 allele were considered *APOE4* positive (*APOE4+*). Further logistic regression analyses using these models and predictors were completed with MD as an outcome. In the model where MD was the outcome of interest, owing to a smaller case number, only the full 14 years of follow-up was considered.

Additional chi-square and t-tests were carried out to compare participants with and without Aβ misfolding by the included covariates. Levels of C-reactive protein (CRP) as a marker of inflammation were also compared between Aβ groups, where CRP levels were classified as a binary variable (high, $\geq$3 mg/L).

Receiver operating characteristic (ROC) curve analysis was completed for both Aβ misfolding and *APOE4* status, where Aβ misfolding and *APOE4* status were considered as binary variables as in the logistic regression analyses previously described. Further supplementary ROC curve analysis was completed where Aβ misfolding was considered continuously and *APOE* was considered categorically (*APOE* ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4) with *APOE* ε3/ε3 as the reference. Area under the ROC curves (AUCs) for AD diagnosis were calculated based on *APOE4* status, Aβ misfolding status, and Aβ and *APOE4* together. ROC contrast analysis was conducted to compare for significant differences between curves.

All analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC). Statistical tests were two sided and conducted at an α-level of 0.05.

3 | RESULTS

3.1 | Participant characteristics

The nested case-control study used for these analyses comprised 59 AD cases, 57 VD cases, 34 MD cases, and 620 controls (Fig. 1). AD was diagnosed within 8 years after baseline in 24 cases and between 8 and 14 years in an additional 35 cases. Main characteristics of study participants are shown in Table 1 and additional information regarding MD and combined dementia in Supplementary Table 1. The mean age at recruitment was 69 years for participants diagnosed with AD, 68 for VD, 70 for MD, and 68 for controls. The age range of included participants at baseline was 52 to 75 years. The majority were female

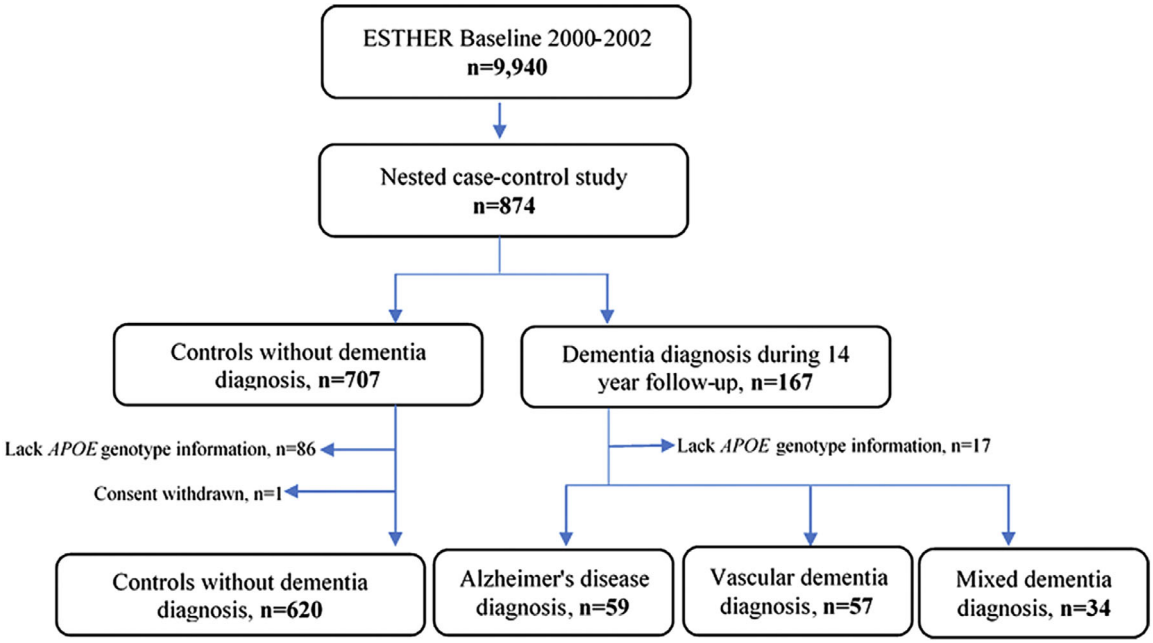

FIGURE 1 Flowchart of ESTHER study participants included in analyses

(59%, 65%, and 58%, respectively) except in MD cases (41%) and had completed 9 years or less of school education (88%, 94%, 81%, and 88% respectively). Aβ misfolding, defined as the previously described threshold indicating Aβ misfolding, was rare among controls (11%), VD cases (11%), and MD cases (12%), but very common among both cases who were diagnosed with AD within 8 years (75%) or between 8 and 14 years (71%) after recruitment. *APOE4* positivity was also more common among AD cases (46% overall) than among VD cases (32%), MD cases (21%), and controls (25%) but differences were less pronounced than differences in Aβ.

There were no significant differences between participants with or without presence of Aβ misfolding in any of the included covariates including age or high CRP levels ($P = .44$).

3.2 | Alzheimer's disease prediction

APOE4+ participants showed a significantly higher odds of AD diagnosis compared to *APOE4*− participants between 8 and 14 years and the complete 14 years of follow-up ($OR_{8-14yrs,}$ 95% CI: 2.9, 1.3–6.4; OR_{14yrs}, 95% CI: 2.4, 1.3–4.7) (Table 2). However, stronger associations were seen between Aβ misfolding and the presence of AD diagnosis in all tested groups independent of the duration of follow-up (OR_{8yrs}, 95% CI: 27.0, 10.0–73.2; $OR_{8-14yrs,}$ 95% CI: 22.1, 9.6–50.5; OR_{14yrs}, 95% CI: 23.0, 11.8–44.5). None of the other demographic (age, sex, education), lifestyle (physical activity), or health risk factors (obesity, diabetes, hypertension, cardiovascular events, depression) were significantly predictive of AD diagnosis.

The disease prediction accuracy as measured by the AUC value of *APOE4* status, Aβ misfolding status, and both predictors combined after 14 years of follow-up was 0.61, 0.81, and 0.85, respectively (Fig. 2). There were significant differences between all ROC curves where the AUC of the combination of *APOE4* and Aβ status was significantly greater than Aβ ($P = .03$) or *APOE4* ($P < .0001$) alone, and the AUC of Aβ status was also significantly greater than *APOE4* status ($P < .0001$). The resulting AUC values using *APOE* as a categorical variable, Aβ misfolding status as a continuous variable, and combined were 0.64, 0.83, and 0.86, respectively (Supplementary Fig. 1).

3.3 | Vascular, mixed, and combined dementia prediction

The only risk factor that significantly predicted VD was the history of cardiovascular events (OR, 95% CI: 2.5, 1.2–5.0) (Table 2). Male sex was predictive of MD (OR, 95% CI: 2.4, 1.1–5.0) (Supplementary Table 2). Both Aβ and *APOE4* status did not express significantly greater odds of VD or MD (Table 2 and Supplementary Table 2). This remained true for VD diagnosed during the first 8 years and between 8 and 14 years of follow-up (Supplementary Table 3). Aβ misfolding was also predictive of any dementia (AD + VD + MD) throughout the 14 years of follow-up and *APOE4* of dementia diagnosed between 8 and 14 years and during the entire 14 years of follow-up (Supplementary Table 2).

4 | DISCUSSION

In this prospective community-based cohort study, Aβ misfolding state showed a strong ability to predict clinical diagnosis of AD over a follow-up of 14 years and far exceeded the predictive ability of *APOE4* status or other dementia risk factors, even 8–14 years before diagnosis. These results suggest that AD risk stratification based on

TABLE 1 Participant characteristics

Characteristic	AD cases (0–8 years)	AD cases (8–14 years)	AD cases (0–14 years)	VD cases (0–14 years)	Controls	*P* value*	*P* value†
n	24	35	59	57	620		
Aβ misfolding, n (%)	18 (75.0)	25 (71.4)	43 (72.9)	6 (10.5)	68 (11.0)	<.0001	.92
APOE4 +, n (%)	10 (41.7)	17 (48.6)	27 (45.8)	18 (31.6)	152 (24.5)	<.001	.24
APOE ε2/ε2, n (%)	0	0	0	0	5 (0.8)		
APOE ε2/ε3, n (%)	3 (12.5)	4 (11.4)	7 (11.9)	8 (14.0)	92 (14.8)		
APOE ε2/ε4, n (%)	0	0	0	4 (7.0)	24 (3.9)	<.001	.61
APOE ε3/ε3, n (%)	11 (45.8)	14 (40.0)	25 (42.4)	31 (54.4)	371 (59.8)		
APOE ε3/ε4, n (%)	9 (37.5)	16 (45.7)	25 (42.4)	14 (24.6)	119 (19.2)		
APOE ε4/ε4, n (%)	1 (4.2)	1 (2.9)	2 (3.4)	0	9 (1.5)		
Sex, n (% female)	14 (58.3)	21 (60.0)	35 (59.3)	37 (64.9)	358 (57.7)	.81	.29
Age, mean (range) at baseline	68.21 (58–75)	69.20 (59–75)	68.80 (58–75)	68.23 (52–75)	68.40 (53–75)	.53	.80
Education, n (% ≤9 years)	21 (87.5)	31 (88.6)	52 (88.1)	*n* = 54 51 (94.4)	*n* = 616 542 (88.0)	.97	.15
Physical activity‡					*n* = 619	.48	.64
Inactive, n (%)	8 (33.3)	14 (40.0)	22 (37.3)	21 (36.8)	191 (30.9)		
Low, n (%)	10 (41.7)	14 (40.0)	24 (40.7)	25 (43.9)	301 (48.6)		
Moderate/high, n (%)	6 (25.0)	7 (20.0)	13 (22.0)	11 (19.3)	127 (20.5)		
Obesity, n (%)	5 (20.8)	4 (11.4)	9 (15.3)	16 (28.1)	*n* = 600 167 (27.8)	.04	.97
Diabetes, n (%)	7 (29.2)	9 (25.7)	16 (27.1)	13 (22.8)	*n* = 618 120 (19.4)	.16	.54
Hypertension, n (%)	14 (58.3)	21 (60.0)	35 (59.3)	41 (71.9)	422 (68.1)	.17	.55
Cardiovascular events, n (%)	2 (8.3)	*n* = 32 2 (6.3)	*n* = 56 4 (7.1)	*n* = 56 14 (25.0)	*n* = 589 75 (12.7)	.22	.01
Depression, n (%)	*n* = 23 3 (3.0)	7 (20.0)	*n* = 58 10 (17.2)	*n* = 53 7 (13.2)	*n* = 583 74 (12.7)	.33	.91

Abbreviations: Aβ, amyloid beta; *APOE4* +, ≥1 ε4 allele; AD, Alzheimer's disease; VD, vascular dementia; n, number of participants.

*P value for comparison between AD cases (14 years) and controls.

†P value for comparison between VD cases and controls.

‡Physical activity (PA): inactive, <1 hr PA/week; low, 1–2 hrs PA/week; moderate/high, ≥2 hrs PA/week.

TABLE 2 Prediction of Alzheimer's disease and vascular dementia diagnosis during 14 years of follow-up: Results of multiple logistic regression

	AD (0–8 years)		AD (8–14 years)		AD (0–14 years)		VD (0–14 years)	
Predictor	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**
Aβ misfolding	**25.74* (9.77–67.81)**	**27.08* (10.03–73.15)**	**21.65* (9.84–47.65)**	**22.05* (9.62–50.53)**	**23.07* (12.19–43.63)**	**22.95* (11.84–44.47)**	1.01 (0.41–2.45)	1.02 (0.41–2.53)
APOE4 +	2.20 (0.96–5.06)	1.99 (0.78–5.09)	**2.91† (1.46–5.80)**	**2.88† (1.30–6.38)**	**2.60† (1.51–4.49)**	**2.44† (1.27–4.68)**	1.41 (0.78–2.54)	1.38 (0.76–2.51)
Male sex	0.96 (0.42–2.22)	0.82 (0.30–2.24)	0.96 (0.48–1.94)	0.81 (0.34–1.93)	0.96 (0.56–1.67)	0.85 (0.42–1.71)	0.73 (0.41–1.30)	0.66 (0.36–1.20)
Age (per year)	0.99 (0.91–1.08)	0.99 (0.89–1.09)	1.04 (0.96–1.12)	1.03 (0.94–1.13)	1.02 (0.96–1.08)	1.02 (0.95–1.10)	0.99 (0.93–1.05)	0.98 (0.92–1.04)
Education ≥10 years	1.07 (0.31–3.67)	1.59 (0.40–6.38)	0.92 (0.32–2.69)	1.21 (0.36–4.03)	0.98 (0.43–2.24)	1.36 (0.52–3.57)	0.50 (0.16–1.55)	0.46 (0.13–1.62)
PA low	0.78 (0.29–2.07)	0.52 (0.17–1.59)	0.66 (0.30–1.45)	0.45 (0.18–1.13)	0.70 (0.38–1.32)	0.55 (0.26–1.16)	0.82 (0.44–1.53)	0.77 (0.41–1.45)
PA mod/high	1.11 (0.36–3.40)	0.74 (0.20–2.66)	0.79 (0.30–2.08)	0.53 (0.17–1.62)	0.91 (0.43–1.92)	0.63 (0.26–1.57)	0.86 (0.39–1.90)	0.88 (0.40–1.94)
Obesity	0.69 (0.25–1.89)	0.82 (0.25–2.70)	0.35 (0.12–1.00)	0.46 (0.14–1.53)	0.48 (0.23–1.00)	0.65 (0.27–1.54)	1.00 (0.54–1.84)	0.93 (0.49–1.77)
Diabetes	1.73 (0.70–4.27)	2.00 (0.66–6.08)	1.40 (0.64–3.08)	1.36 (0.51–3.61)	1.53 (0.83–2.82)	1.63 (0.75–3.53)	1.22 (0.63–2.34)	1.16 (0.59–2.31)
Hypertension	0.66 (0.29–1.53)	0.65 (0.24–1.79)	0.66 (0.33–1.35)	0.73 (0.31–1.71)	0.66 (0.38–1.15)	0.71 (0.36–1.41)	1.21 (0.66–2.22)	1.11 (0.58–2.10)
Cardiovascular events	0.63 (0.15–2.72)	0.52 (0.10–2.77)	0.52 (0.13–2.02)	0.51 (0.10–2.72)	0.56 (0.20–1.55)	0.50 (0.15–1.73)	**2.54 (1.29–5.00)**	**2.49 (1.23–5.02)**
Depression	1.04 (0.30–3.61)	1.09 (0.25–4.67)	1.76 (0.73–4.21)	2.14 (0.72–6.41)	1.45 (0.70–3.01)	1.59 (0.62–4.10)	0.97 (0.41–2.27)	0.83 (0.35–1.96)

NOTE. Model 1: adjusted for age, sex, and education. Model 2: adjusted for all included covariates.

Statistically significant results at *P* value < .05 are bolded.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; *APOE4* +, ≥1 ε4 allele; CI, confidence interval; OR, odds ratio; VD, vascular dementia; PA, physical activity: inactive (reference) ≤1 hr PA/week, low = 1–2 hrs PA/week, moderate(mod)/high ≥2 hrs PA/week.

*P value < .0001.

†P value < .01

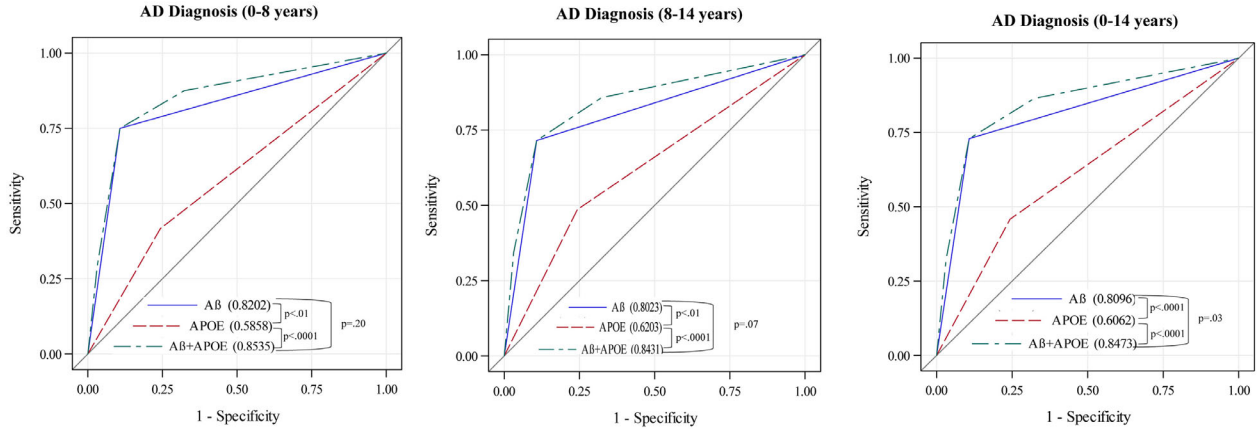

FIGURE 2 Alzheimer's disease (AD) diagnosis prediction based on *APOE4* status, Aβ misfolding status, and both predictors together. AD diagnosis prediction was examined in three groups, Follow-up after 8 and 14 years and between 8 and 14 years. Portrayed above are area under the curve (AUC) values and *P* values from receiver operating characteristic (ROC) curve contrast analyses between predictors. Specific AUC and 95% confidence intervals (CIs) for the AD predictors were [AUC (95% CI)]: Aβ status: 0–8 yrs, 0.82 (0.73–0.91), 8–14 yrs, 0.80 (0.73–0.88), 0–14 yrs, 0.81 (0.75–0.87); *APOE4* status: 0–8 yrs, 0.59 (0.48–0.69), 8–14 yrs, 0.62 (0.53–0.71), 0–14 yrs, 0.61 (0.54–0.67); Aβ + *APOE4* status: 0–8 yrs, 0.85 (0.77–0.94), 8–14 yrs, 0.84 (0.77–0.92), 0–14 yrs, 0.85 (0.79–0.90).

Aβ misfolding in plasma could be a promising strategy for identifying individuals at greater risk of AD in both the short and long term in therapeutic/preventative treatment development and enhanced risk stratification.

Although it has been recommended that risk stratification be based on more accurate neuropathological changes,[5,7,9] several current large-scale AD prevention studies have targeted individuals based on AD genetic risk (*APOE4* status).[7] Individuals with one and two *APOE4* allele(s) have a 3- and 15-fold increased risk of AD, respectively.[29] Even though the prevalence of carriage of two *APOE4* alleles is much lower than the prevalence of Aβ misfolding (in our study: 1.5% vs. 11.0% among controls) and carriers of two *APOE4* alleles comprise a very high risk group, the risk associated with Aβ misfolding was found to be stronger than *APOE4* status in our study (OR, 23.0, 11.8–44.5). Additional risk stratification strategies and prevention studies have also been based on demographic, health, and lifestyle factors.[7,30,31] The presented risk stratification strategy based on Aβ misfolding in blood plasma expressed greater long-term disease prediction accuracy than *APOE4* status or other health and lifestyle risk factors and may therefore be useful in targeting individuals for future research.

Other large-scale AD prevention studies have targeted individuals with amyloid positivity determined through CSF-derived biomarkers or PET imaging.[7] Although CSF-derived and PET imaging are the accepted gold standard to detect AD pathological changes in the brain, these techniques are either costly or invasive, where blood is readily available and plasma assays more affordable. While the goal of our study was the use of plasma Aβ misfolding as an AD risk identifier and not a diagnostic tool for individual clinical care, it is still important to compare with established biomarkers to have an idea of its performance and possible place in future research. In the clinical setting, CSF-derived biomarkers have shown high disease prediction accuracy with previous studies reporting AUC values ranging from 0.87 to 0.96 when comparing AD cases to healthy controls[10] and PET imaging has also expressed high accuracy for cortical amyloidosis, with a sensitivity and specificity of 96% and 100% for detecting moderate to frequent plaques.[11] However, CSF requires a lumbar puncture by a trained physician[32] and PET imaging is expensive.[32] Again, it is important to note these studies have illustrated the ability of these tools to diagnose AD or identify prodromal AD in a clinical setting.[10,33] A risk identifier such as Aβ misfolding in plasma could present an alternative for risk stratification.

Recently, several AD blood biomarkers have been developed and validated, which determine Aβ pathology in the brain.[14,15,34,35] In a study by Nakamura et al.,[16] a blood-based Aβ biomarker showed high accuracy in predicting brain amyloid-β burden; however, it should be taken into account that these results are compared only to PET imaging ($AUC_{discovery}$ = 0.97 and $AUC_{validation}$ = 0.94) and not clinical diagnosis as in this study. It is to be expected that blood biomarkers will also be tested longitudinally in large epidemiologic cohorts to assess their potential as risk factors in clinical settings and in the general population. Although early plasma studies (before more sophisticated assays) did include several refined cohort studies, consistent associations to AD were not evident.[15,36–38] More recently, plasma amyloid measured by enzyme-linked immunosorbent assays was shown to be associated to AD progression in the Rotterdam study.[39]

Finally, it is important to consider that although Aβ in plasma originates from cells of the liver, kidney, muscle, and lung, Aβ in peripheral plasma can also derive from the central nervous system through active and passive Aβ clearance mechanisms or impaired blood-brain barrier functions. Using the immuno-infrared sensor, we could not identify the direct origin of plasma Aβ but have previously observed an increased proportion of β-sheet-enriched isoforms similar to Aβ in CSF in AD cases.[24] Furthermore, the lack of relationship between Aβ misfolding and age in our study or previous studies[19,24,25] and inflammation measured by high CRP levels at baseline in our study asserts its disease-specific nature and that it is not simply a symptom of normal aging.

4.1 | Implications

The measurement of pathological changes rather than risk based on genetic, lifestyle, or health factors is critical in the development of effective therapeutic disease-modifying treatments targeted toward AD.[1] Individuals at high AD risk identified through plasma could receive additional PET imaging and/or CSF examination while low-risk individuals could be immediately excluded thereby reducing costs, improving participant experience, and increasing ease. Furthermore, those at high AD risk could potentially be targeted in the future for enhanced preventative strategies including multidomain intervention programs incorporating lifestyle factors and vascular risk management.[31]

4.2 | Strengths and limitations

The findings presented in this study are a robust comparison between AD risk factors and Aβ misfolding in blood plasma,[19] investigated longitudinally in a community-based cohort with an extensive follow-up period of 14 years. These results provide an insightful picture of the proficiency of Aβ misfolding as a risk factor for AD diagnosis in a community population with implications for future enhanced AD risk stratification.

It is important to note several limitations including: (1) the possibility of dementia misdiagnosis/underdiagnosis both among cases and controls; (2) the possibility of delayed dementia diagnosis due to community setting and relatively low level of education; (3) the inability to complete stratified analysis based on sex, age, and education level due to sample size; (4) the possibility that Aβ-misfolding may occur in some individuals without AD and might be lacking in some individuals with AD; and (5) the relatively small sample of AD/VD cases which might have led to nonsignificant results of known risk factors for AD and VD as well as the relatively large confidence intervals. The presence of misdiagnosis or underdiagnosis of dementia may have led to an underestimation of the Aβ misfolding-AD association: possible nondifferential misclassification of some AD cases as VD cases would not be expected to bias the association of Aβ misfolding with the remaining AD cases, but nondifferential misclassification of VD cases as AD cases would be expected to lead to underestimation of the Aβ misfolding-AD association. Furthermore, underdiagnosis may have led to underestimation as it is possible that some of the controls may develop dementia later or have not been diagnosed with dementia although present, which would again be expected to lead to an underestimation of associations.

5 | CONCLUSION

Aβ misfolding measured in blood plasma was a strong risk prediction marker for AD clinical diagnosis even many years before clinical manifestation in a community-based sample followed over 14 years, but not for clinical diagnosis of MD or VD, and may present a promising complement to *APOE4* status or other dementia risk factors in AD risk stratification in the therapeutic development setting. The strong predictive value of Aβ misfolding should be confirmed in larger population-based studies.

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Authors' contributions: H.S., A.N., L.P., K.G., and H.B. made substantial contributions to the concept and design, interpreting data, and drafting/revising the manuscript. A.N. performed the immuno-infrared analyses. H.S. carried out epidemiological analyses. T.M. contributed to epidemiological analyses and B.S. to the coordination of the ESTHER study. L.P., B.S., B.H., D.R., and H.B. contributed to data acquisition for the ESTHER study. A.H. contributed to the interpretation of data. K.G. conceived the immuno-infrared sensor for secondary structure analysis of protein misfolding. H.B. conceived and led the ESTHER study. All authors revised the manuscript for important intellectual content and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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