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RESEARCH ARTICLE

Aβ misfolding in blood plasma measured by immuno-infrared-sensor as an age-independent risk marker of Alzheimer's disease

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Abstract

Introduction: Determining potential risk factors of amyloid beta (Aβ) misfolding in blood, a risk marker for clinical Alzheimer's disease (AD), could have important implications for its utility in future research and clinical settings.

Methods: Participants aged 50 to 75 years attending a general health examination were recruited for a prospective community-based cohort study in Saarland, Germany, in 2000 to 2002. For these analyses, participants with available Aβ misfolding measurements and clinical AD information at 17-year follow-up were included ($n = 444$).

Results: Age did not show any association with Aβ misfolding in plasma; however, a strong association of both age and Aβ misfolding with the incidence of clinical AD was evident. Education and cardiovascular diseases were likewise not associated with Aβ misfolding.

Discussion: Structural measurement of Aβ misfolding in blood plasma is an age-independent risk marker of clinical AD among older adults, supporting that risk of clinical AD is already largely determined before older adulthood.

KEYWORDS
age, Alzheimer's disease, amyloid beta misfolding, cohort study, risk factors

1 | INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by amyloid beta (Aβ) deposits and tau tangles in the brain.[1] Clinical diagnosis of AD is made when dementia symptoms become manifest, which may occur decades after neuropathologies are present. To determine clinical AD risk at an early stage, it is important to focus on markers of AD pathological changes, as they may occur many years before clinical symptoms.[1,2] In 2018, the National Institute on Aging and Alzheimer's Association (NIA-AA) recommended a shift in the definition of AD as a biological construct (presence of Aβ and tau).[1,2] To develop effective intervention and prevention measures for clinical AD, it is necessary to assess factors that are associated with pathological changes of AD.

Pathological change of AD includes structural changes of the Aβ peptide, also known as misfolding, thus altering its folds from healthy monomeric predominantly disordered or partially α-helical to pathological β-sheet-enriched secondary structures.[3] These β-sheet-enriched structures aggregate, and can form soluble toxic oligomers and macroscopically visible amyloid plaques, which are thought to contribute to AD neurodegeneration.[4,5] However, misfolding causes a shift in the overall secondary structure distribution within the total Aβ fraction in cerebrospinal fluid (CSF) and blood plasma. One strategy to measure structural misfolding of Aβ in blood plasma is using a novel immuno-infrared-sensor (iRS).[6,7] Using this technique, we have previously shown that Aβ misfolding in blood plasma is correlated to CSF AD biomarkers and amyloid positron emission tomography (PET) imaging and is highly predictive of AD diagnosis many years before clinical AD diagnosis.[7,8] These findings suggest that Aβ misfolding in blood plasma is an early risk marker of clinical AD risk and may be a marker of early AD pathological change.

While clinical AD has many varying modifiable and non-modifiable risk factors including genetic predisposition and cardiovascular disease, the greatest risk factor is age. Like AD incidence, Aβ blood concentration markers have also been shown to increase with age.[9] To what extent Aβ misfolding as a structural AD risk marker is also age dependent or may be present earlier in life possibly even prior to any increase in Aβ blood concentration is unknown, however. As an age-independent marker it could potentially be useful for targeted, risk-adapted AD prevention.

Therefore, the aim of this study was to assess the association of age and other clinical AD risk factors with Aβ misfolding, a structural marker of AD pathological change, within a community-based cohort study of older adults. The association between age and these risk factors and AD incidence was investigated in parallel for comparison.

HIGHLIGHTS

- Amyloid beta (Aβ) misfolding is strongly associated with clinical Alzheimer's disease (AD).
- Aβ misfolding in blood is unrelated to age among older adults suggesting that risk of clinical AD is already largely determined before older adulthood.
- Education is not associated with Aβ misfolding, supporting the cognitive reserve theory.

RESEARCH IN CONTEXT

1. **Systematic review**: Recently, amyloid beta (Aβ) misfolding in blood has been identified as a marker that strongly predicts Alzheimer's disease (AD) years before occurrence of the disease. It has been unclear, however, how early in life such misfolding can be detected and to what extent it is related to AD risk factors.
2. **Interpretation**: In this study among older adults, we did not find any association between age and Aβ misfolding in blood, suggesting that it is an age-independent risk marker among older adults and that risk of clinical AD is already largely determined before older adulthood. The absence of an association between education and Aβ misfolding supports the cognitive reserve theory.
3. **Future directions**: Investigations of the longitudinal relationship between early-life and further genetic risk factors, and Aβ misfolding as well as an assessment of the progression of Aβ misfolding over time and in adults younger than 60 years of age are needed.

2 | METHODS

2.1 | Study design and population

The analyses were conducted among participants of the ongoing community-based prospective ESTHER cohort study (German: Epidemiologische Studie zu Chancen der Verhütung Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung).[7,10,11] In short, participants aged 50 to 75 years attending a general health examination were recruited by their general practitioners (GPs) in a statewide study in Saarland, Germany in 2000 to 2002. Participants filled in standardized self-administered health questionnaires and provided blood samples, including heparin plasma samples, which were stored at −80°C. Further medical information was provided by GPs and comprehensive follow-ups were conducted 2, 5, 8, 11, 14, and 17 (ongoing) years after recruitment. Information on vital status and causes of death was obtained from population registries and local health authorities. 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 ESTHER study includes 9940 participants. ESTHER participants with available dementia diagnosis information and Aβ misfolding measurements were included in analyses. Information regarding clinical AD

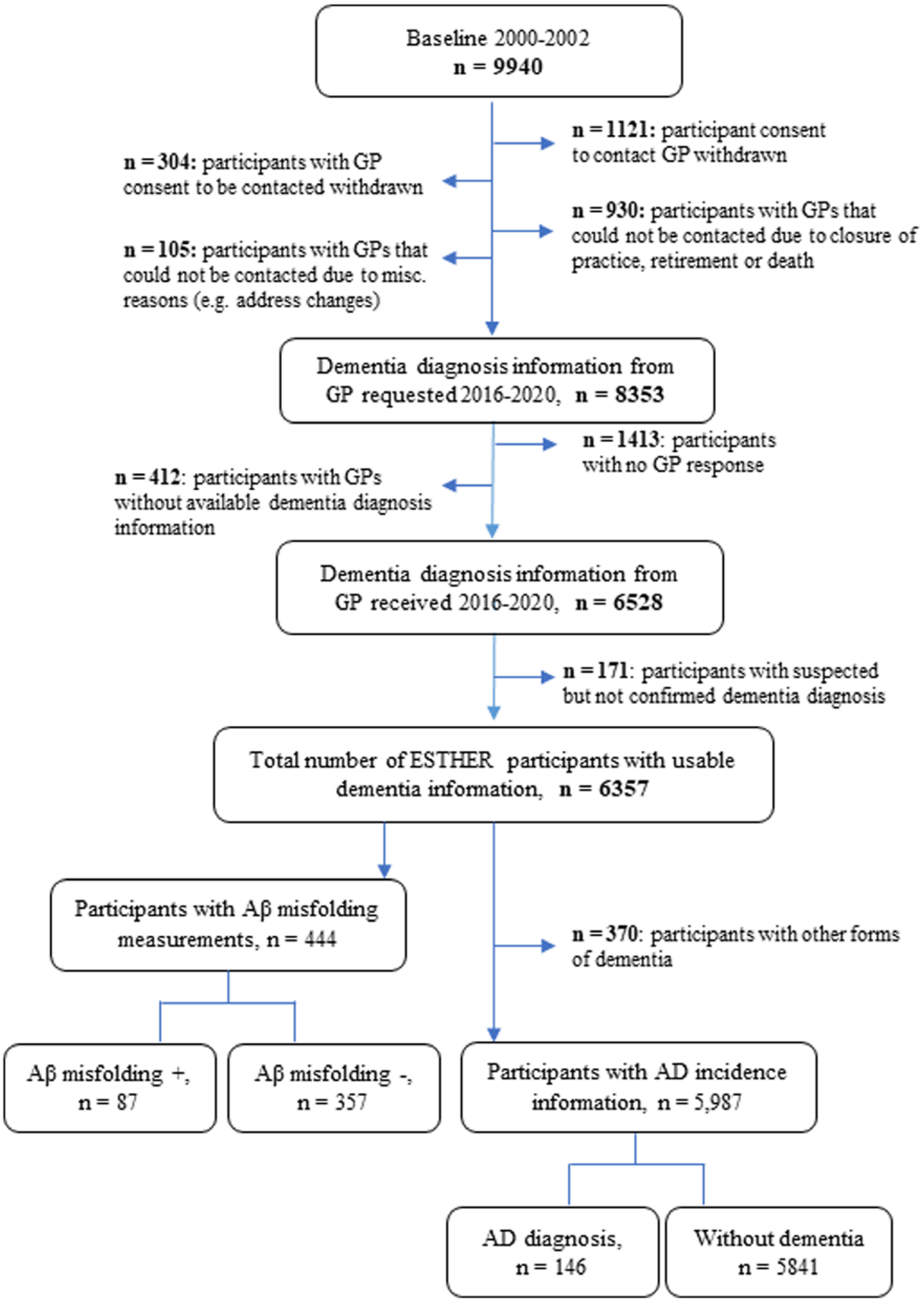

FIGURE 1 Participants from the ESTHER prospective cohort study included in analyses

diagnosis and lack of dementia diagnosis were collected from participants' GPs during the 14- and 17-year ESTHER follow-ups as previously reported.[11] Briefly, all GPs of all ESTHER participants were contacted at the 14- and 17-year follow-ups and asked to fill out a detailed questionnaire regarding dementia diagnoses of their patients as well as to provide all available medical records of neurologists, psychiatrists, memory, or other specialized providers. The current guidelines in Germany for AD diagnosis follow the NIA-AA[12] or the International Working group (IWG)-2 criteria.[13,14] Overall, 5987 participants with available information regarding AD diagnosis or confirmed lack of dementia diagnosis were included (Figure 1).

2.2 | Biomarkers and covariates

The blood plasma samples used in this study were collected at baseline as previously reported in detail.[7] Briefly, soluble Aβ peptides were completely extracted from baseline blood plasma and alterations in

the Aβ peptide secondary structure distribution were measured for each participant with the novel iRS (WO 2015121339 A1).[6,7] In agreement with the previously validated spectral threshold,[7] participants with a cutoff of <1642 cm^{-1} were considered to have increased Aβ misfolding.

Apolipoprotein E (*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). Participants with ≥1 *APOE* ε4 allele were considered *APOE* ε4 positive (*APOE* ε4+).

Risk factors of AD ascertained at baseline included age, sex, educational level, *APOE* ε4, and several cardiovascular diseases. Educational level was measured by years of formal education (≤9 years or >9 years; the lower category corresponds to a leaving certificate from school or less and the higher to more education than the minimum expected in the German school system). The following cardiovascular diseases were assessed: hypertension (physician diagnosis or use of anti-hypertensive drugs), myocardial infarction (physician diagnosis), stroke (physician diagnosis), coronary heart disease (physician diagnosis), and heart failure (physician diagnosis). In addition, the number of cardiovascular diseases was summed and modeled as a dichotomous and continuous variable.

2.3 | Statistical methods

This study consisted of two analyses: (1) the main cross-sectional analyses investigating the association between age and clinical AD risk factors with Aβ misfolding measured in blood and (2) for comparison, the Cox proportional hazard analysis investigating the association between the above-mentioned risk factors and incidence of clinical AD within 17 years of follow-up.

In the main cross-sectional analyses, 19% of participants had at least one missing information item of the variables included in this study. Therefore, multiple imputations for data missing at random with 19 imputations were done using the Markov chain Monte Carlo (MCMC) method[15] and analyses completed with the imputed datasets. Multiple logistic regression, using Aβ misfolding status as the dependent variable, was used to calculate crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) for each risk factor. Adjusted analyses included the covariates: age, sex, education, *APOE* ε4, and a variable indicating dementia case or control status.

In the Cox proportional hazards analyses, 16% of participants had at least one missing value in the variables included in this study and multiple imputations for data missing at random with 16 imputations was done using the MCMC method.[15] The censoring dates for these analyses included date of AD diagnosis, date of death, date of dropout, or date of the 17-year follow-up (date of response from the GP regarding dementia diagnosis status). Cox proportional hazards regression was used to calculate crude and adjusted hazard ratios (HRs) including 95% CIs that were calculated for each of the previously mentioned risk factors with incidence of clinical AD diagnosis as the main outcome. Adjusted analyses included the covariates age, sex, education, and *APOE* ε4 status. All analyses were conducted using SAS software, version 9.4 (SAS Institute). A significant statistical difference was defined by P values < .05 in two-sided testing.

3 | RESULTS

3.1 | Participant characteristics

Details regarding the participant characteristics and a flowchart outlining the sample derivation are presented in Table 1 and Figure 1.

A total of 444 participants were included in the main cross-sectional Aβ misfolding analyses (Figure 1). Of these, 87 participants had increased Aβ misfolding present in blood plasma and 357 were considered Aβ misfolding negative (or lacking increased Aβ misfolding in blood plasma). The mean age of those with increased Aβ misfolding was 68 years and those without was also 68 years. A total of 37% of participants with increased Aβ misfolding present were *APOE* ε4+ compared to 28% of those who were Aβ misfolding negative. There were more females (58%) among Aβ misfolding negative participants compared to participants with increased Aβ misfolding present (55%). Distributions of additional clinical AD risk factors by Aβ misfolding status can be found in Table 1.

Of the 444 participants included in the main analyses, 68 participants received a clinical diagnosis of AD within 17 years of follow-up, respectively (Table S1 in supporting information). A total of 376 participants remained without dementia diagnosis within 17 years. Prevalence of Aβ misfolding was 11% among controls, compared to 62.3% among participants who were diagnosed with AD during follow-up (Table S1). The distribution of Aβ misfolding according to AD status and age group at baseline is additionally depicted in Table S1. No increase in Aβ misfolding prevalence with age could be seen, neither in AD cases, nor in other dementia cases, nor in participants without dementia diagnoses.

A total of 5987 participants were included in the secondary AD incidence analyses (Figure 1 and Table 1). Of these, 146 participants received a clinical AD diagnosis and 5841 remained without dementia diagnosis within 17 years of follow-up. The mean age of those diagnosed with clinical AD and those without dementia diagnosis was 67 and 61 years at baseline, respectively. A larger proportion of participants without dementia diagnosis (67%) was observed in the 50 to 64 age group at baseline, compared to only 33% of those diagnosed with AD. About half (49%) of participants that were diagnosed with AD were *APOE* ε4+ compared to only 25% in those that remained without dementia diagnosis. More females (61%) were among those diagnosed with AD compared to 55% of those without dementia. Additionally, a greater proportion (82%) of participants diagnosed with AD had <9 years of formal education compared to 73% of those with dementia diagnosis. Additional participant characteristics according to AD status are presented in Table 1.

TABLE 1 Participant characteristics

Characteristic		ESTHER participants with Aβ misfolding measurements		ESTHER participants with AD information from 17-year follow-up	
		Aβ misfolding$^+$ *n* (%)	Aβ misfolding$^-$ *n* (%)	AD diagnosis, *n* (%)	Participants without dementia, *n* (%)
Total		87 (19.6)	357 (80.4)	146 (2.4)	5841 (97.6)
Age at baseline	Mean ± SD	68.2 ± 4.8	68.0 ± 4.6	66.7 ± 5.1	61.3 ± 6.5
	50–64	17 (19.5)	77 (21.5)	48 (32.9)	3902 (66.8)
	65–69	27 (31.0)	127 (35.6)	45 (30.8)	1259 (21.6)
	70–75	43 (49.4)	153 (42.9)	53 (36.3)	680 (11.6)
Sex	Female	48 (55.2)	206 (57.7)	89 (61.0)	3184 (54.5)
	Male	39 (44.8)	151 (42.3)	57 (39.0)	2657 (45.5)
Education	≤9 years	79 (90.8)	309 (86.8)	117 (82.4)	4154 (72.7)
	≥10 years	8 (9.2)	47 (13.2)	25 (17.6)	1561 (27.3)
APOE ε4+	No	52 (62.7)	225 (72.4)	65 (50.0)	3926 (75.0)
	Yes	31 (37.4)	86 (27.7)	65 (50.0)	1309 (25.0)
Hypertension	No	32 (36.8)	121 (33.9)	56 (38.4)	2700 (46.4)
	Yes	55 (63.2)	236 (66.1)	90 (61.6)	3124 (53.6)
Myocardial infarction	No	79 (94.1)	312 (91.5)	135 (96.4)	5387 (95.7)
	Yes	5 (6.0)	29 (8.5)	5 (3.6)	304 (5.3)
Stroke	No	78 (92.9)	324 (96.1)	136 (95.8)	5516 (97.2)
	Yes	6 (7.1)	13 (3.9)	6 (4.2)	161 (2.8)
Coronary heart disease	No	65 (74.7)	286 (80.1)	136 (95.8)	5516 (97.2)
	Yes	22 (25.3)	71 (19.9)	6 (4.2)	161 (2.8)
Heart failure	No	74 (85.1)	290 (82.9)	131 (89.7)	5236 (90.1)
	Yes	13 (14.9)	60 (17.1)	15 (10.3)	574 (9.9)
Number of cardiovascular diseases	0-2	74 (85.1)	313 (87.7)	136 (93.2)	5434 (93.0)
	>2	13 (14.9)	44 (12.3)	10 (6.9)	407 (7.0)

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; *APOE*, apolipoprotein E; SD, standard deviation.

3.2 | The association among age, other AD risk factors, and Aβ misfolding

The results of the cross-sectional logistic regression analyses assessing the association between clinical AD risk factors and Aβ misfolding are presented in Table 2. There was no association between age and Aβ misfolding, neither when age was coded as a variable with three categories (OR 65–69: 0.80, 95% CI 0.37–1.73; OR 70–75: 1.01, 95% CI 0.49–2.09), nor when it was included as a continuous variable (OR 0.98, 95% CI 0.72–1.33 per 5-year increase in age). Additionally, there was no association present between years of formal education and Aβ misfolding (OR for >9 compared to ≤9 years: 0.47, 95% CI 0.18–1.21).

There were no statistically significant associations for hypertension (OR 0.99, 95% CI 0.55–1.80), myocardial infarction (OR 0.96, 95% CI 0.32–2.87), stroke (OR 1.83, 95% CI 0.54–6.16), and heart failure (OR 1.04, 95% CI 0.49–2.20) in regard to Aβ misfolding. Furthermore, the number of cardiovascular diseases was not associated with Aβ misfolding, neither as a dichotomous (OR 1.80, 95% CI 0.82–3.96) nor as a continuous variable (OR 1.16, 95% CI 0.89–1.52). However, a statistically significant association was evident between coronary heart disease and Aβ misfolding (OR 2.05, 95% CI 1.05–3.99).

3.3 | The association between clinical AD incidence and AD risk factors including age

The results of the Cox proportional hazards regression analyses among 5987 ESTHER participants with information regarding AD diagnosis or lack of dementia diagnosis throughout 17 years of follow-up are shown in Table 3. A strong relationship between age and incidence of clinical AD was evident. Participants that were in the age groups 65 to 69 years and 70 to 75 years at baseline were diagnosed with clinical AD 3.2 and 8.5 times more frequently than those participants aged 50 to 64 years at baseline (HR 65–69: 3.20, 95% CI 2.13–4.81; HR 70–75: 8.51, 95% CI 5.74–12.63). A comparison of the magnitude of association between age at baseline and Aβ misfolding and incidence of clinical AD is depicted in Figure 2.

TABLE 2 Distribution of sample characteristics and cross-sectional association to Aβ misfolding: results of multiple logistic regression

Characteristic		N_{total} (col %)	N_{Abeta+} (row %)	OR (95% CI)		*P* value*
				Crude	Adjusted[a]	
Age	50–64	94 (21.2)	17 (18.1)	Ref.	Ref.	
	65–69	154 (34.7)	27 (17.5)	0.96 (0.49–1.88)	0.80 (0.37–1.73)	.5704
	70–75	196 (44.1)	43 (21.9)	1.27 (0.68–2.38)	1.01 (0.49–2.09)	.9768
	Per 5 years	444 (100)	87 (19.6)	1.08 (0.83–1.41)	0.98 (0.72–1.33)	.8804
Sex	Female	254 (57.2)	48 (18.9)	Ref.	Ref.	
	Male	190 (42.8)	39 (20.5)	1.11 (0.69–1.78)	1.46 (0.83–2.57)	.1862
Education	≤9 years	388 (87.6)	79 (20.4)	Ref.	Ref.	
	≥10 years	55 (12.4)	8 (14.6)	0.66 (0.30-1.46)	0.47 (0.18-1.21)	.1158
APOE ε4+	No	277 (70.3)	52 (18.8)	Ref.	Ref.	
	Yes	117 (29.7)	31 (26.5)	1.60 (0.97–2.66)	1.07 (0.58–1.96)	.8387
Hypertension	No	153 (34.5)	32 (20.9)	Ref.	Ref.	
	Yes	291 (65.5)	55 (18.9)	0.88 (0.54–1.44)	0.99 (0.55–1.80)	.9861
Myocardial infarction	No	391 (92.0)	79 (20.2)	Ref.	Ref.	
	Yes	34 (8.0)	5 (14.7)	0.70 (0.27–1.85)	0.96 (0.32–2.87)	.9486
Stroke	No	402 (95.5)	78 (19.4)	Ref.	Ref.	
	Yes	19 (4.5)	6 (31.6)	1.82 (0.67–4.97)	1.83 (0.54–6.16)	.3278
Coronary heart disease	No	351 (79.1)	65 (18.5)	Ref.	Ref.	
	Yes	93 (21.0)	22 (23.7)	1.36 (0.79–2.36)	**2.05 (1.05–3.99)**	**.0351**
Heart failure	No	364 (83.3)	74 (20.3)	Ref.	Ref.	
	Yes	73 (16.7)	13 (17.8)	0.84 (0.44–1.60)	1.04 (0.49–2.20)	.9138
Number of cardiovascular diseases	0–2	387 (87.2)	74 (19.1)	Ref.	Ref.	
	>2	57 (12.8)	13 (22.8)	1.25 (0.64–2.44)	1.80 (0.82–3.96)	.1456
	Continuous	444 (100)	87 (19.6)	1.01 (0.81–1.27)	1.16 (0.89–1.52)	.2752

Note: Bolded results indicate achievement of statistical significance, $P < .05$.

[a]Adjusted for age, sex, education, *APOE* ε4, and case/control status.

*P value derived from multiple logistic regression adjusted model.

Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; CI, confidence interval; OR, odds ratio.

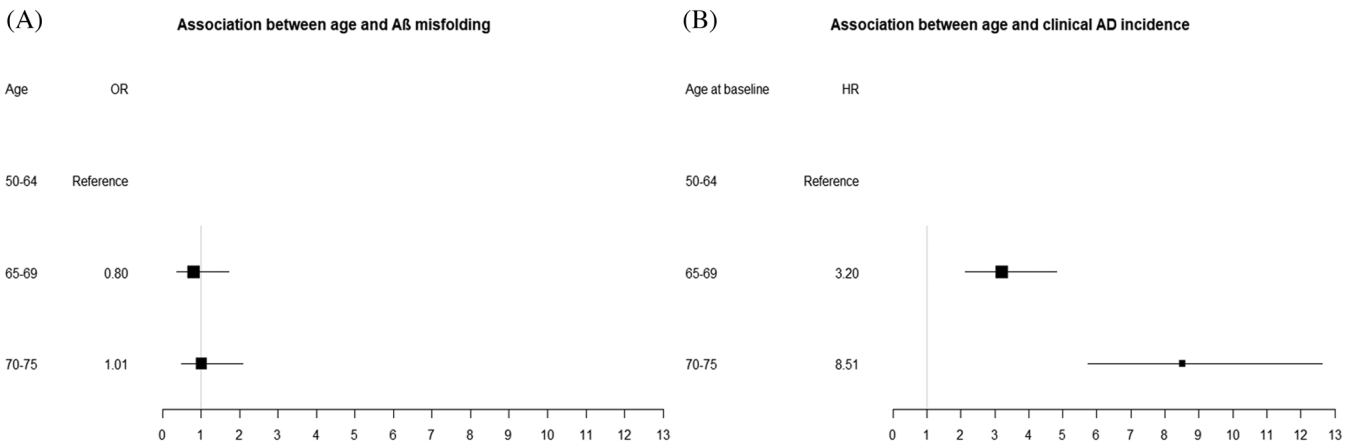

FIGURE 2 Association of age at baseline with (A) Aβ misfolding at baseline and with (B) incidence of clinical AD diagnosed throughout 17 years of follow-up. The reference group for both analyses is the group aged 50 to 64 years at baseline. Aβ, amyloid beta; AD, Alzheimer's disease; HR, hazard ratio; OR, odds ratio.

TABLE 3 Distribution of sample characteristics at baseline and association to incidence of clinical Alzheimer's disease diagnosis throughout 17 years of follow-up: results of Cox proportional hazards regression

Characteristic		N_{total} (col %)	N_{AD} (row %)	HR (95% CI) Crude	HR (95% CI) Adjusted[a]	*P* value*
Age at baseline	50–64	3950 (66.0)	48 (1.2)	Ref.	Ref.	
	65–69	1304 (21.8)	45 (3.5)	**3.20 (2.13–4.81)**	**3.20 (2.13–4.81)**	**<.0001**
	70–75	733 (12.2)	53 (7.2)	**8.02 (5.42–11.88)**	**8.51 (5.74–12.63)**	**<.0001**
	Per 5 y	5987 (100)	146 (2.4)	**2.25 (1.93–2.63)**	**2.30 (1.96–2.69)**	**<.0001**
Aβ misfolding	No	357 (80.4)	22 (6.2)	Ref.	Ref.	
	Yes	87 (19.6)	46 (52.9)	**11.48 (6.90–19.12)**	**11.21 (6.67–18.85)**	**<.0001**
Sex	Female	3273 (54.7)	89 (2.7)	Ref.	Ref.	
	Male	2714 (45.3)	57 (2.1)	0.82 (0.59–1.14)	0.85 (0.61–1.19)	.3435
Education	≤9 years	4271 (72.9)	117 (2.7)	Ref.	Ref.	
	≥10 years	1586 (27.1)	25 (1.6)	**0.55 (0.36–0.84)**	**0.58 (0.38–0.90)**	**<.0140**
APOE ε4+	No	3990 (74.4)	64 (1.6)	Ref.	Ref.	
	Yes	1373 (25.6)	64 (4.7)	**2.96 (2.10–4.18)**	**3.20 (2.27–4.52)**	**<.0001**
Hypertension	No	2756 (46.2)	56 (2.0)	Ref.	Ref.	
	Yes	3214 (53.8)	90 (2.8)	**1.48 (1.06–2.07)**	1.06 (0.75–1.49)	.7499
Myocardial infarction	No	5522 (94.7)	135 (2.4)	Ref.	Ref.	
	Yes	309 (5.3)	5 (1.6)	0.85 (0.35–2.04)	0.56 (0.23–1.35)	.1951
Stroke	No	5652 (97.1)	136 (2.4)	Ref.	Ref.	
	Yes	167 (2.9)	6 (3.6)	1.83 (0.81–4.15)	1.48 (0.65–3.37)	.3526
Coronary heart disease	No	5263 (88.0)	125 (2.4)	Ref.	Ref.	
	Yes	721 (12.1)	21 (2.9)	1.39 (0.88–2.21)	0.91 (0.57–1.47)	.7071
Heart failure	No	5367 (90.1)	131 (2.4)	Ref.	Ref.	
	Yes	589 (9.9)	15 (2.6)	1.19 (0.69–2.02)	0.80 (0.47–1.38)	.4234
Number of cardiovascular diseases	0–2	5570 (93.0)	136 (2.4)	Ref.	Ref.	
	>2	417 (7.0)	10 (2.4)	1.15 (0.61–2.19)	0.74 (0.38–1.42)	.3632
	Continuous	5987 (100)	146 (2.4)	**1.19 (1.01–1.40)**	0.96 (0.81–1.15)	.6714

Note: Bolded results indicate achievement of statistical significance, $P < .05$.

[a]Adjusted for age, sex, education, and *APOE ε4* status.

**P* value derived from Cox regression with adjustment for covariates.

Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; CI, confidence interval; HR, hazard ratio.

Participants were less frequently diagnosed with clinical AD in those that had 10 or more years of formal education compared to those with <9 years (HR 0.58, 95% CI 0.38–0.90). Additionally, participants that had one more *APOE ε4* allele(s) were 3.2 times more frequently diagnosed with clinical AD compared to those without any *APOE ε4* allele. Furthermore, Aβ misfolding was strongly associated with clinical AD (HR 11.21, 95% CI 6.67–18.85). None of the additional risk factors reached statistical significance (Table 3).

4 | DISCUSSION

In this study assessing the relationship between age and other clinical AD risk factors and Aβ misfolding in blood plasma, a structural AD risk marker measured by iRS, age was not associated to Aβ misfolding, despite its strong association with AD incidence. Aβ misfolding in plasma appears to be an age-independent risk factor of clinical AD and may have important implications on future clinical AD risk assessment.

4.1 | Age and Aβ misfolding

The absence of an association of age with Aβ misfolding in the ESTHER study is in sharp contrast to greater incidence of clinical AD with increasing age. Age is known to be one of the greatest risk factors of clinical AD[16] and has been associated to blood concentration levels of Aβ1-40 and Aβ1-42, and Aβ cerebral burden.[9,17–20] Furthermore, age has been able to predict AD-pattern neurodegeneration.[20,21] The diagnostic accuracy of CSF Aβ levels has been shown to be age dependent as well.[22] Our findings, however, support that Aβ misfolding is a

disease-specific marker and not an effect of aging. Previous cohorts in which Aβ misfolding has been measured also exhibited a lack of association to age.[6,7] Although not the focus of these previous studies, this trend is evident and asserts the robust nature of our findings in this study. The iRS-based Aβ misfolding measurement is unique in that it discerns structural changes of Aβ. It is thought that the pathological change, including the structural change of Aβ from healthy disordered and/or α helical to pathological β-sheet-enriched secondary structures, occurs 15 to 20 years before clinical onset of AD.[3,23,24] Hence, it could be speculated that at a mean baseline age of 68 years as in our study the pathological change has already occurred for most participants who will go on to develop AD. Furthermore, it could be assumed that Aβ misfolding in blood is a marker of susceptibility to clinical AD risk. In a previous analysis we were able to show a strong association of Aβ misfolding with clinical AD within 14 years of follow-up.[8] As evident in the updated analysis, a very strong association of Aβ misfolding with clinical AD remains within 17 years of follow-up (HR 11, $P < .0001$). However, to clarify the relationship between Aβ misfolding and age, longitudinal analyses at different time-points as well as measurements among younger adults are necessary.

Furthermore, the lack of association between age and Aβ misfolding indicates that clinical risk may be largely determined before late adulthood. While the time of progression between pathological changes and clinical AD symptoms may be influenced by additional medical, lifestyle, and cognitive reserve factors,[25] Aβ misfolding may be more heavily influenced by early/mid-life and genetic determinants. Several studies have shown an association between *APOE* and AD polygenic risk scores and the concentration of Aβ measured in CSF, through PET, and by *post mortem* examination.[26–28] A limited number of genome-wide association studies has investigated Aβ accumulation.[29] In the future, larger studies may provide more insight into the biological mechanism behind Aβ structural change and accumulation. Additionally, other earlier life risk factors should be explored, such as early/mid-life cardiovascular and neurovascular health, as they may play a role in Aβ structural change before late adulthood.

4.2 | Other AD risk factors and Aβ misfolding

In accordance with the cognitive reserve theory, which suggests that a high educational level does not protect from AD pathology but it rather delays cognitive decline,[30,31] a higher education was not associated with Aβ misfolding. This result is also in line with a study showing that educational level is not associated with cerebrospinal markers of AD pathology but it is positively correlated with brain functional network efficiency.[32]

With regard to cardiovascular diseases, we did not observe a significant association toward an increased risk for Aβ misfolding, with the exception of coronary heart disease. This might be due to the cross-sectional design of the study and due to the examination of late-life risk factors as opposed to mid-life risk factors as well as small sample size. However, there have been conflicting results regarding mid-life vascular risk factors and their association with brain Aβ deposition.[33,34] It should also be noted that this is the first study examining the association of cardiovascular diseases with Aβ misfolding, a structural rather than concentration marker of Aβ in plasma. Nevertheless, there have been studies showing that (cardio-) vascular risk factors are not associated with brain Aβ deposition and hence might also not be involved in Aβ misfolding.[35–37] Instead, cardiovascular diseases might enhance neurodegeneration once Aβ is accumulated.[21,38]

4.3 | Strengths and limitations

A limitation of this study was the small simple size, which might have prevented the observation of significant effects. Also, the cross-sectional approach in the main analyses prevents any conclusion about temporality or causality of associations. Another limitation of the study includes the possibility of dementia misdiagnosis/underdiagnosis. The dementia diagnoses made in the ESTHER study were clinical diagnoses reported heterogeneously by numerous practitioners, which reflects the process and quality of diagnoses in outpatient settings. This fundamental difference to clinical based cohorts in specialized academic settings can be assumed to have led to inferior diagnostic accuracy. However, this is the nature of community-based cohort studies that portray common practice in such a setting. Nevertheless, the very strong associations with Aβ misfolding, which were selectively seen for participants with AD diagnoses but not for participants with other types of dementia, support reasonable validity of the clinical diagnoses.[7,8] Additionally, dementia neuropathologies are complex where AD pathology seldom occurs in isolation,[39] further complicating diagnoses.

Strengths of this study include community-based data, which reflect representative clinical settings, the use of medical diagnoses, and the novel assessment of the relationships between risk factors of AD and Aβ misfolding in plasma.

5 | CONCLUSION

This study focused on the relationship between clinical AD risk factors and Aβ misfolding, an early marker of clinical AD risk measured by iRS in blood plasma and discerns structural changes in Aβ. Our results indicate that Aβ misfolding is an age-independent risk factor of clinical AD in older adulthood, asserting that clinical AD risk may be largely determined before older adulthood.

Future studies with larger sample sizes should investigate the longitudinal relationship between early-life and further genetic risk factors, and Aβ misfolding to discover potential for intervention and prevention measures as well as to provide more insight into AD pathogenesis. Additionally, an assessment of the progression of Aβ misfolding over time and in adults younger than 60 years of age is needed.

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CONFLICTS OF INTEREST

The secondary structure-based, Aβ misfolding marker measured by the iRS is protected by one approved patent (EP3324187B1) and three patent applications (WO 2015121339 A1, WO 2018091743 A1, and WO 2018219969 A1) by KG and AN.

AUTHOR CONTRIBUTIONS

TM, HS, LP, and HB made substantial contributions to the concept and design, interpreting data, and drafting the manuscript. AN performed the immuno-infrared analyses. TM and HS carried out epidemiological analyses. BS contributed to the coordination of the ESTHER study. LP, BS, BH, DR, and HB contributed to data acquisition for the ESTHER study. AMH contributed to the interpretation of data. KG conceived the immuno-infrared-sensor for secondary structure analysis of protein misfolding. HB 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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