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Association of Nonmodifiable Risk Factors With Alzheimer Disease Blood Biomarkers in Community-Dwelling Adults in the ESTHER Study

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Abstract

Background and Objectives

Dementia-related blood biomarkers are the future of large-scale dementia risk stratification; however, the extent to which phosphorylated tau (P-tau181), neurofilament light (NfL), and glial fibrillary acidic protein (GFAP) are associated with nonmodifiable risk factors has yet to be confirmed in the community, and the role of menopause has yet to be investigated. Therefore, the aim of this study was to examine the association of age, sex, *APOEe4* status, and menopause, with dementia-related blood biomarker levels (P-tau181, NfL, and GFAP) and rate of change over 11 years in longitudinal biomarker measurements in community-dwelling adults.

Methods

Within this German population-based Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung cohort study (n = 9,940), a nested case-control study of 1,026 participants (1:1, without dementia during follow-up: incident dementia during follow-up) aged 50–75 years at baseline followed over 17 years was conducted. Blood biomarker measurements (P-tau181, NfL, and GFAP) were completed in blood from baseline, 8-year, and 11-year follow-ups, and cross-sectional and longitudinal regression analyses were used to assess the association with age, sex, *APOEe4*, and menopause.

Results

The mean age of participants was 64 years, and women accounted for slightly over half (54%) of the sample. Age was cross-sectionally and longitudinally significantly associated with all dementia-related biomarkers ($p < 0.001$). NfL and GFAP levels more strongly correlated (Spearman $R = 0.55$ and 0.49) with age at baseline than P-tau181 levels (Spearman $R = 0.21$). Women experienced significantly higher levels and rates of increase in GFAP ($p < 0.001$) while men experienced higher levels of NfL after adjusting for age and *APOEe4* ($p < 0.01$). *APOEe4* status was significantly associated with baseline and longitudinal levels of P-tau181 (baseline $\beta = 0.30$, $p < 0.05$) and GFAP (baseline $\beta = 15.84$, $p < 0.001$). Of interest, premenopausal status was significantly associated with higher GFAP levels after adjusting for age, sex, and *APOEe4* ($\beta = 19.09$, $p < 0.05$).

Discussion

This population-based study on dementia biomarkers found that P-tau181 was dependent on age and *APOEe4*; NfL on age and sex; and GFAP on age, sex, *APOEe4*, and menopause status. GFAP levels and rate of increase were higher in women, especially in premenopausal participants. Future research should confirm these findings and further explore the role of menopause in dementia pathogenesis among women.

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Glossary

AD = Alzheimer disease; **ESTHER** = Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung; **GFAP** = glial fibrillary acidic protein; **GP** = general practitioner; **IWG** = International Working Group; **NfL** = neurofilament light; **P-tau181** = phosphorylated tau.

Introduction

Blood biomarkers for Alzheimer disease (AD) and related dementias are essential to the future of AD prescreening and characterization in older adults, which are critical for the development and success of future preventive and disease-modifying strategies.[1,2] Blood-based phosphorylated tau (P-tau181) is an established marker of AD pathology that can predict Aβ and tau pathologies and identify AD across the continuum and may be used in the future to confirm probable AD.[3] Neurofilament light (NfL) chain levels in blood reflect nondisease-specific neurodegeneration and could aid in tracking disease progression in the clinical setting.[4] Glial fibrillary acidic protein (GFAP) in blood is a measure of astrocyte activation and may be a very early biomarker of AD pathology.[1] GFAP is a reflection of reactive astrogliosis, and it remains unclear whether the biomarker is an AD-specific marker.[2]

Extensive research has been conducted to establish these blood biomarkers as diagnostic and prognostic markers in a clinical setting,[2] and less research has, however, focused on the measurement in the community setting and the influence of nonmodifiable factors, such as age, sex, *APOEe4*, and menopause. The greatest risk factors for development of AD and dementia are age and genetics driven mostly by *APOEe4*.[5,6] Furthermore, it is known that women are affected at greater rates by AD and dementia compared with men.[7] However, the association of menopause with dementia-related blood biomarkers has not yet been investigated, which might provide important information regarding dementia risk among women.

While it has been shown that NfL is quite age dependent,[8] the extent to which P-tau181 and GFAP are dependent on age is less clear and the literature regarding the sex specificity of the biomarkers is mixed.[2] In an at-risk population followed for up to 4 years, age, sex, and *APOEe4* status were shown to differentially affect P-tau181, NfL, and GFAP rate of change.[9] The influence of these nonmodifiable risk factors especially menopause in a population-based cohort with a long follow-up, to our knowledge, is yet to be investigated. This is important to inform age-specific, sex-specific, and *APOE*-specific recommendations for risk stratification and to aid in answering critical questions regarding the role of nonmodifiable risk factors including menopause and sex hormones in disease pathogenesis.

Therefore, the aim of this study was to examine the association of nonmodifiable risk factors, age, sex, and *APOEe4* status, with AD-related blood biomarker levels (P-tau181, NfL, and GFAP) and rate of change in longitudinal biomarker measurements in community-dwelling adults aged 50–75 years at baseline who were followed over 17 years within the prospective population-based Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung (ESTHER) study. Secondarily, the effects of menopause on biomarker levels were examined.

Methods

Study Participants and Data Collection

These analyses are derived from a nested case-control study within the ESTHER study, a population-based prospective cohort study of community-dwelling older adults in Germany. In brief, the ESTHER study initially enrolled 9,940 participants, who were recruited by general practitioners (GPs) during a health screening visit across the state of Saarland, a federal state in southwestern Germany with a population of approximately 1 million residents, between 2000 and 2002. The inclusion criteria for the ESTHER study encompassed individuals aged 50–75 years, with the ability to speak and read German to provide written informed consent.[10-12]

Data on dementia diagnoses were collected from participants' GPs, who were asked to fill out questionnaires and provide all available medical records regarding dementia diagnoses (or lack of dementia diagnoses) throughout follow-up. The current guidelines in Germany for AD diagnosis follow the National Institute on Aging and the Alzheimer's Association[13] or the International Working Group (IWG)-2 criteria; for vascular dementia diagnosis, the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria[14]; and for mixed dementia diagnosis, the IWG criteria for mixed dementia.[15-17] More details regarding the ESTHER study and the dementia diagnoses can be found in eAppendix 1.

The sample for this study is a nested case-control sample including all participants who had been diagnosed with dementia between baseline and the 17-year follow-up (n = 513) and randomly selected controls (n = 513; participants confirmed by own GPs to be without dementia diagnosis throughout follow-up) (Figure 1). Longitudinal measurements of biomarkers were completed for participants who had available blood samples at the 8-year and 11-year follow-ups. Complete cases were used in cross-sectional and longitudinal analyses.

Standard Protocol Approvals, Registrations, and Patient Consents

The ESTHER study was approved by the ethics committee of the Medical Faculty at Heidelberg University and the Physicians' Board of Saarland, and written informed consent from all participants was obtained. The report of this study followed the Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline.

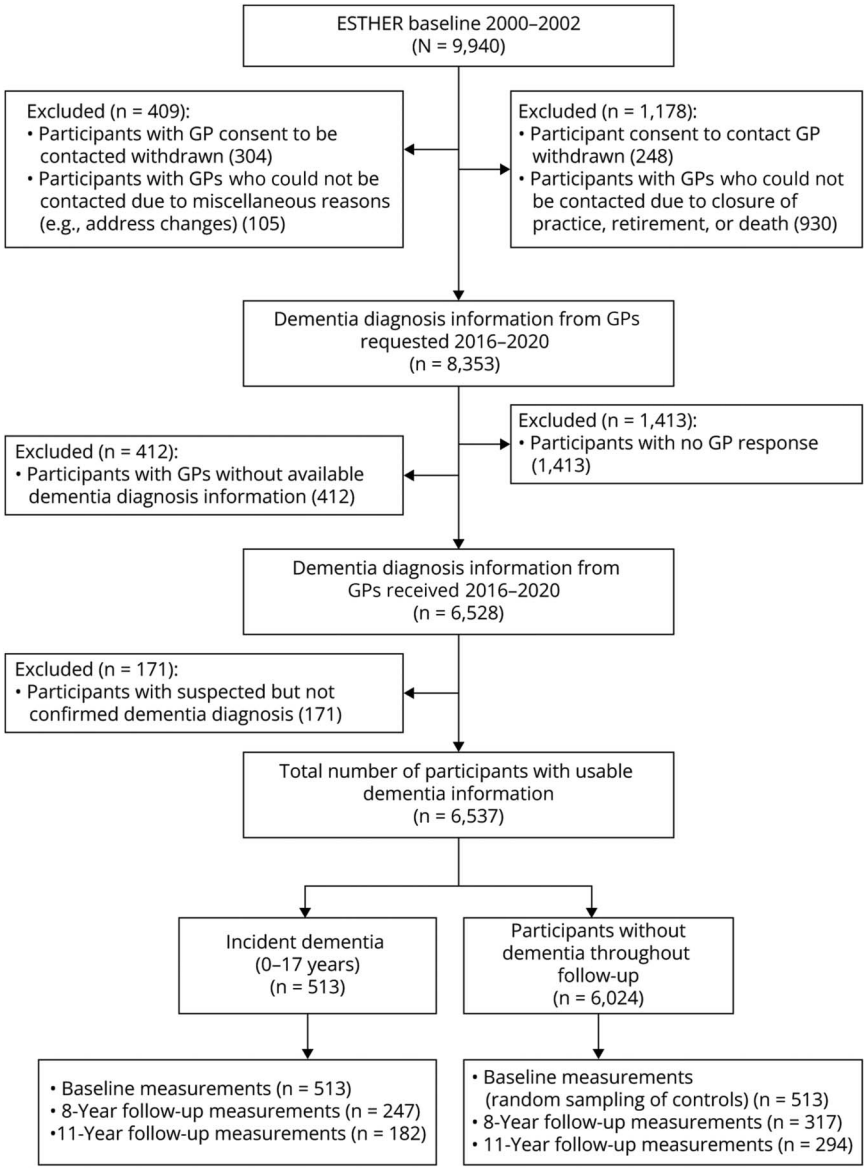
Data Ascertainment at Baseline and Laboratory Measurements

Demographic, medical, and lifestyle data were collected at baseline using self-administered questionnaires and/or physician reports. The baseline questionnaire did not include a specific item on race or ethnicity. However, European descent was determined through genetic analysis during genetic data quality control.[18] The P-tau181, GFAP, and NfL measurements were completed using the automated Simoa HD-X Analyzer by Quanterix as previously described.[12] Details regarding data ascertainment and laboratory test measurements can be found in eAppendix 2.

Statistical Analysis

Descriptive statistics were used to provide information regarding participant characteristics in the entire sample as well as in incident dementia cases (0–17 years) and in controls (participants who remained without dementia diagnosis throughout follow-up). Mann-Whitney *U* tests were used to assess

Figure 1 Flowchart of the Nested Case-Control Study Including Participants With Dementia-Related Blood Biomarker Measurements



GP = general practitioner.

significant differences in biomarker levels by sex and *APOEe4* carrier status. Spearman rank correlation coefficients were calculated to assess the correlation between age and the dementia-related biomarkers. In the analyses including *APOEe4* and menopause, participants with missing data were excluded from linear regression and linear mixed-effects analyses.

Cross-sectional analyses at baseline were completed to assess the association of P-tau181, NfL, and GFAP (dependent variables) with age, sex, and *APOEe4* (independent variables). In addition, interactions of age at baseline with sex and *APOEe4* status in the association with the biomarkers were examined, and linear regression lines were plotted.

Linear mixed-effects models were used to examine longitudinal person-specific changes in the dementia-related blood biomarkers adjusted for age, sex, and *APOEe4*. Adjusted annual change in P-tau181, NfL, and GFAP levels were reported. Additional linear mixed-effects models including interaction terms (time since baseline in years x independent variable of interest) and covariates age, sex, and *APOEe4* were used to assess whether the rate of biomarker change differed by age, sex, and *APOEe4* carrier status. Linear mixed-effects models were used to account for multiple biomarker measurements per participant over time, and the interaction term including time and independent variable of interest was used to explore whether the rate of change differed according to the non-modifiable risk factors of interest. To assess differences by age, a dichotomous age variable was used according to age at baseline (50–64 years or 65–75 years). Sensitivity analyses included the following: (1) models further adjusted for kidney function measured through eGFR because it is known to affect dementia-related blood biomarkers[17]; (2) models excluding blood biomarker outliers. Outliers were defined as >4 SDs from the mean level at each measurement time point.

All analyses were conducted in the entire sample as well as in participants who developed dementia during follow-up (incident dementia group) and in participants who did not develop dementia during follow-up (controls). Statistical analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC), and R version 4.2.2 in RStudio (2023.03.0). Statistical tests were 2 sided and conducted at an α-level of 0.05.

Data Availability

The data sets generated and/or analyzed during this study are not publicly available because of local regulations but may be made available from the ESTHER study principal investigator on reasonable request with appropriate proposal.

Results

Participant Characteristics

This analysis included 1,026 participants from the ESTHER study (n = 9,940 participants), aged 50–75 years at baseline (mean age: 64 years), and consisted of 54% female participants (Table 1 and Figure 2). The median age at dementia diagnosis was 78 years (interquartile range 74–82 years). *APOEe4* carriers accounted for 39% of the participants in the incident dementia group and 26% of participants who remained without dementia diagnosis (Table 1).

The mean (SD) biomarker levels (pg/mL) were as follows: baseline, P-tau181, 1.78 (2.2), NfL, 17.4 (7.9), and GFAP, 104.2 (63.6); 8-year follow-up, P-tau181, 2.9 (2.9), NfL, 24.1 (24.7), and GFAP, 176.6 (114.3); and 11-year follow up, P-tau181, 3.0 (2.1), NfL, 24.3 (23.7), and GFAP, 174.0 (129.6).

Cross-Sectional Analyses at Baseline

All participants were without dementia diagnosis at baseline. NfL and GFAP levels more strongly correlated (Spearman $R = 0.55$ and 0.49) with age at baseline than P-tau181 levels (Spearman $R = 0.21$) (eFigure 1). Men had significantly higher levels of NfL ($p < 0.05$) while women had significantly higher levels of GFAP at baseline ($p < 0.05$). Finally, *APOEe4* carriers had significantly higher levels of both P-tau181 ($p < 0.05$) and GFAP ($p < 0.001$) (eFigure 1).

Age at baseline was significantly associated with all 3 biomarkers in all groups (all participants, incident dementia group, controls) after adjusting for sex and *APOEe4* status (Figure 2). Being a woman was significantly associated with higher levels of GFAP ($\beta = 10.82$, $p < 0.001$) but lower levels of NfL at baseline ($\beta = 1.69$, $p < 0.01$) after adjustment for age and *APOEe4*. Sex was not associated with P-tau181 levels. Among all participants, *APOEe4* status was significantly associated with both P-tau181 ($\beta = 0.30$, $p < 0.05$) and GFAP ($\beta = 15.84$, $p < 0.001$) levels after adjustment for age and sex. While significant associations remained among participants who developed dementia within 17 years, *APOEe4* status was not significantly associated with any of the biomarkers among participants who remained without dementia throughout follow-up.

When examining the association of age with biomarker levels by sex and *APOEe4* status, no significant interaction by sex was evident. However, the association of age at baseline with P-tau181 and GFAP levels at baseline was modified by *APOEe4* status (Figure 3). Finally, the percentage of the variance explained by age, sex, and *APOEe4* in P-tau181 was 2%, mainly driven by age (1.6%); in NfL was 17% with age as the greatest contributor at 15%; and in GFAP was 16% also with age as the greatest contributor at 13%.

Longitudinal Analyses

The annual rate of change of P-tau181, NfL, and GFAP adjusted for age, sex, and *APOEe4* status among all participants was 0.14, 0.93, and 5.65 pg/mL, respectively (Figure 4).

Age at baseline was significantly associated with change in all biomarkers among all groups (Figure 4). Sex was significantly

Table 1 Summary Statistics by Incident Dementia (0–17 Years After Baseline) Diagnosis

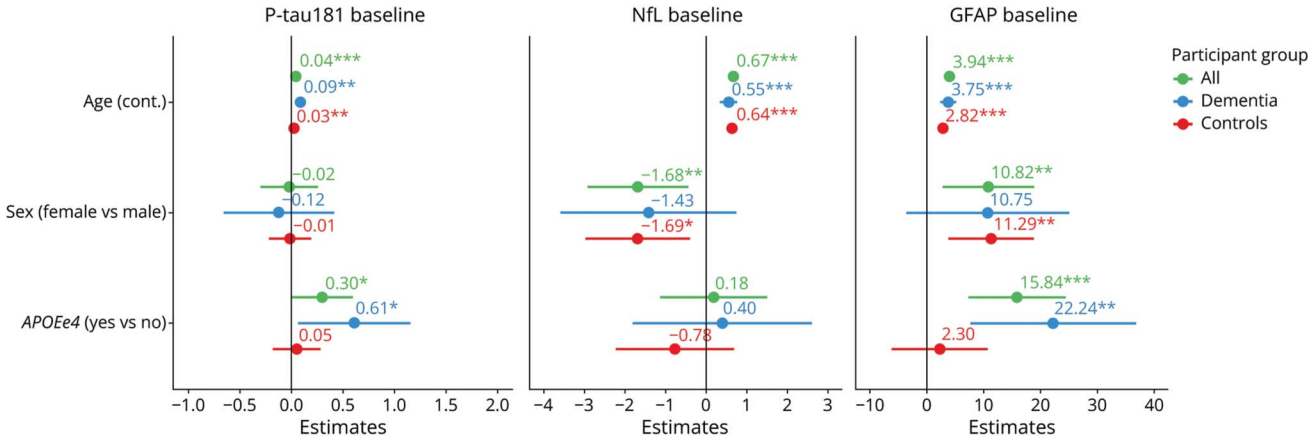
	Control (N = 513)	Dementia (N = 513)	Total (N = 1,026)	*p* Value
Age at baseline, y				<0.001
Mean (SD)	61.27 (6.47)	67.00 (5.13)	64.14 (6.50)	
Range	50.00–75.00	50.00–75.00	50.00–75.00	
Sex, n (%)				0.381
Male	230 (44.8)	244 (47.6)	474 (46.2)	
Female	283 (55.2)	269 (52.4)	552 (53.8)	
***APOEe4*, n (%)**				<0.001
N-Miss	13	31	44	
0 e4 alleles	368 (73.6)	292 (60.6)	660 (67.2)	
≥1 e4 alleles	132 (26.4)	190 (39.4)	322 (32.8)	
Menopause at baseline (women only), n (%)				<0.001
N-Miss	6	6	12	
Yes	246 (88.8)	255 (97.0)	501 (92.8)	
No	31 (11.2)	8 (3.0)	39 (7.2)	

p Values for *t* tests or χ^2 tests assess differences between participants diagnosed with dementia between baseline and 17 years of follow-up and those without dementia diagnosis throughout follow-up.

associated with change in GFAP levels among all participants. Sex was not associated with change in P-tau181 or NfL levels. *APOEe4* was significantly associated with change in P-tau181 and GFAP levels.

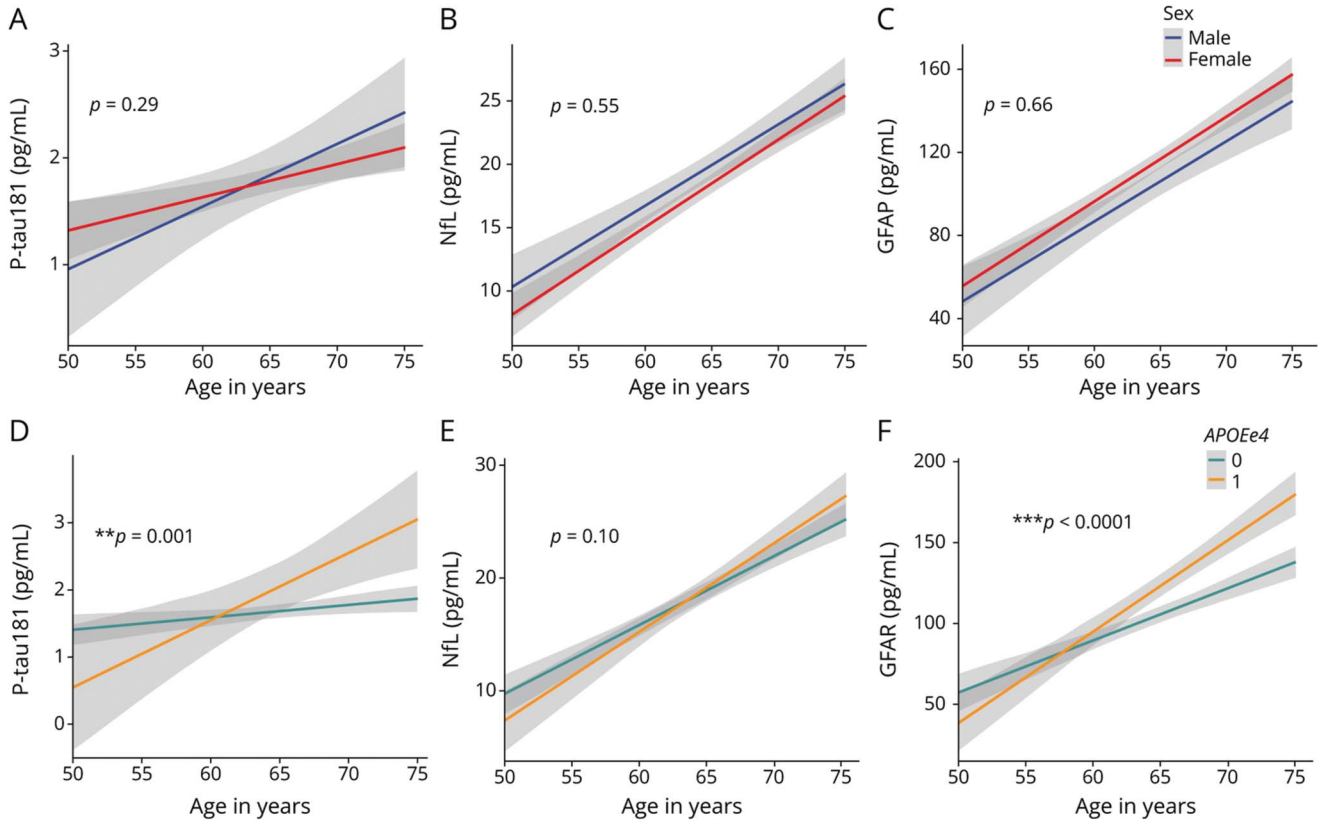
Older participants (65–75 years old at baseline) experienced significantly faster rates of increase in NfL and GFAP levels, but not P-tau181 levels compared with younger participants (50–64 years at baseline) (Figure 5). Among the incident dementia group, older participants experienced significantly faster rates of increase in GFAP levels, whereas among controls, older participants experienced significantly faster rates of increase in NfL levels (eFigures 2 and 3). Women experienced significantly faster rates of increase in GFAP levels among all groups (Figure 5). There was no significant interaction between time and sex or *APOEe4* in the association

Figure 2 Cross-Sectional Linear Regression Results From Baseline



*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Estimates shown are β estimates from linear regression models at baseline. Age: per year increase, sex: female vs male, *APOEe4*: carrier vs noncarrier. Results in green indicate analyses among all participants, in blue among participants who were diagnosed with dementia within 17 years, and in red among participants who were not diagnosed with dementia throughout 17 years of follow-up. GFAP = glial fibrillary acidic protein; NfL =neurofilament light; P-tau181 = phosphorylated tau.

Figure 3 Interaction of Age With Sex and *APOEe4* Status in the Association With Dementia-Related Blood Biomarkers at Baseline



p Values presented are for the interaction between age and sex or *APOEe4* status. **p* < 0.05, ***p* < 0.01, ****p* < 0.001. Age: per year increase, sex: female vs male, *APOEe4*: carrier (1) vs noncarrier (0). GFAP = glial fibrillary acidic protein; NfL =neurofilament light; P-tau181 = phosphorylated tau.

with P-tau181 or NfL levels. *APOEe4* carriers did, however, experience significantly faster rates of increase in GFAP levels among participants who did not develop dementia during follow-up (eFigure 3).

Secondary and Sensitivity Analyses

In the secondary analyses, menopause was significantly associated with GFAP levels but not P-tau181 or NfL levels after adjusting for age and *APOEe4* status (eFigure 4). Women who had not yet gone through menopause had significantly increased GFAP levels compared with postmenopausal participants.

In the sensitivity analyses, eGFR (measure of kidney function) was only significantly associated with cross-sectional and longitudinal NfL levels, but only altered results minimally (eFigure 5). In the second sensitivity analyses, the following numbers of outliers was removed according to the previously defined criteria: baseline: P-tau181 (n = 4), NfL (n = 13), GFAP (n = 6); 8-year follow-up: P-tau181 (n = 11), NfL (n = 10), GFAP (n = 14); 11-year follow-up: P-tau181 (n = 4), NfL (n = 18), GFAP (n = 10). The analyses after removing outliers also did not substantially change any results (eFigures 6 and 7).

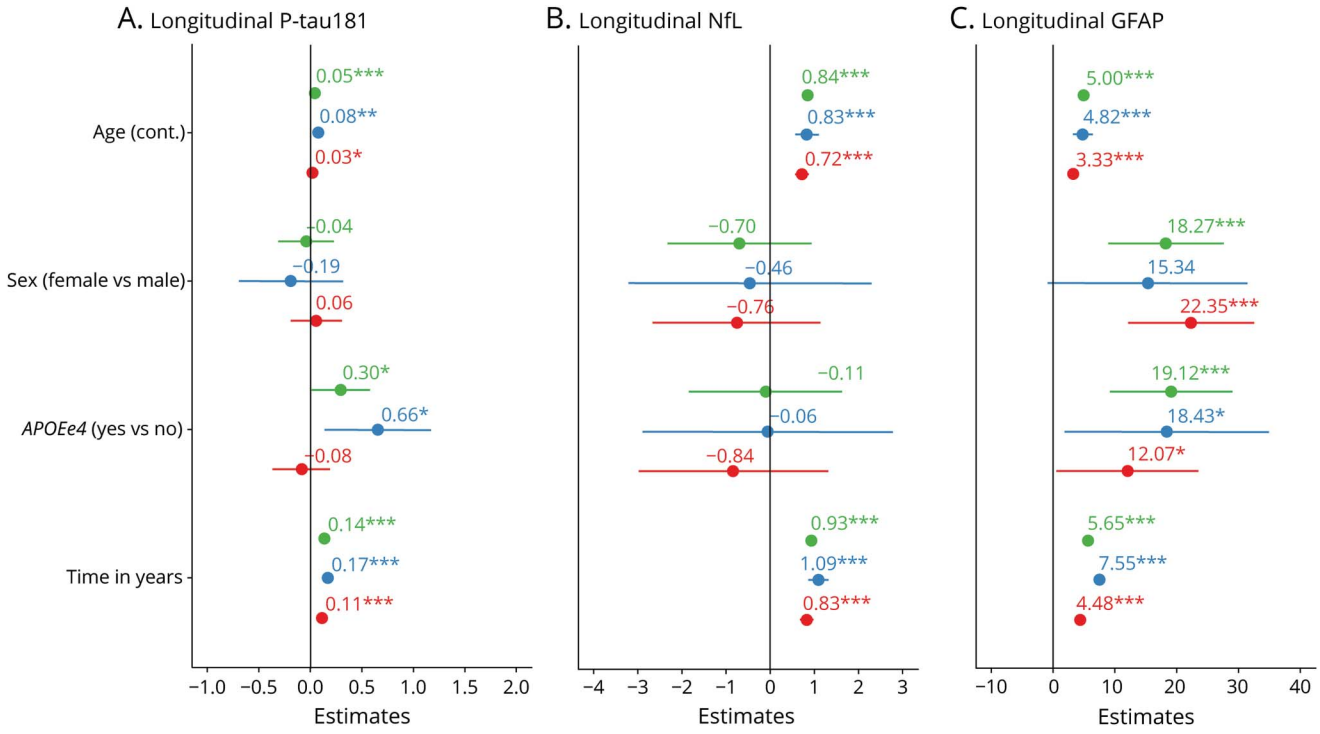
Discussion

In this community-based study of participants followed over 17 years, the age of participants at baseline was associated with cross-sectional and longitudinal levels of the blood biomarkers P-tau181, GFAP, and NfL. Women, especially premenopausal participants, had significantly increased levels of GFAP at baseline and experienced faster rates of increase over time compared with men while men had significantly increased levels of NfL. *APOEe4* carrier status was associated with both GFAP and P-tau181 levels at baseline before dementia diagnosis and longitudinal levels.

It is well known that age is associated with NfL,[2,8,19] but it is less clear whether P-tau181 and GFAP levels are also age-dependent.[2] Previous studies have shown the age-dependent nature of NfL and have even reported age-specific reference values for NfL.[19,20] In addition, in a study of at-risk individuals, participants older than 65 years saw faster increases in NfL levels,[9] as was evident in our study.

Although it is less clear whether P-tau181 and GFAP are age-dependent in the literature,[2] there is some evidence of higher levels of GFAP in older individuals[21] and increased

Figure 4 Linear Mixed-Effects Model Results



*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Estimates shown are β estimates from linear mixed-effect models considering longitudinal levels of P-tau181 (A), NfL (B), and GFAP (C). Age at baseline: per year increase, sex: female vs male, *APOE* e4: carrier vs noncarrier, time in years since baseline (represents increase in biomarker level per year adjusted for age, sex, and *APOEe4*). GFAP = glial fibrillary acidic protein; NfL =neurofilament light; P-tau181 = phosphorylated tau.

levels of P-tau181 beginning in older age.[22] Most previous dementia-related blood biomarker studies included older or at-risk populations while our study included a wide range of adults aged 50–75 years at baseline with greater variation, which may explain age dependency in all biomarkers in our study.

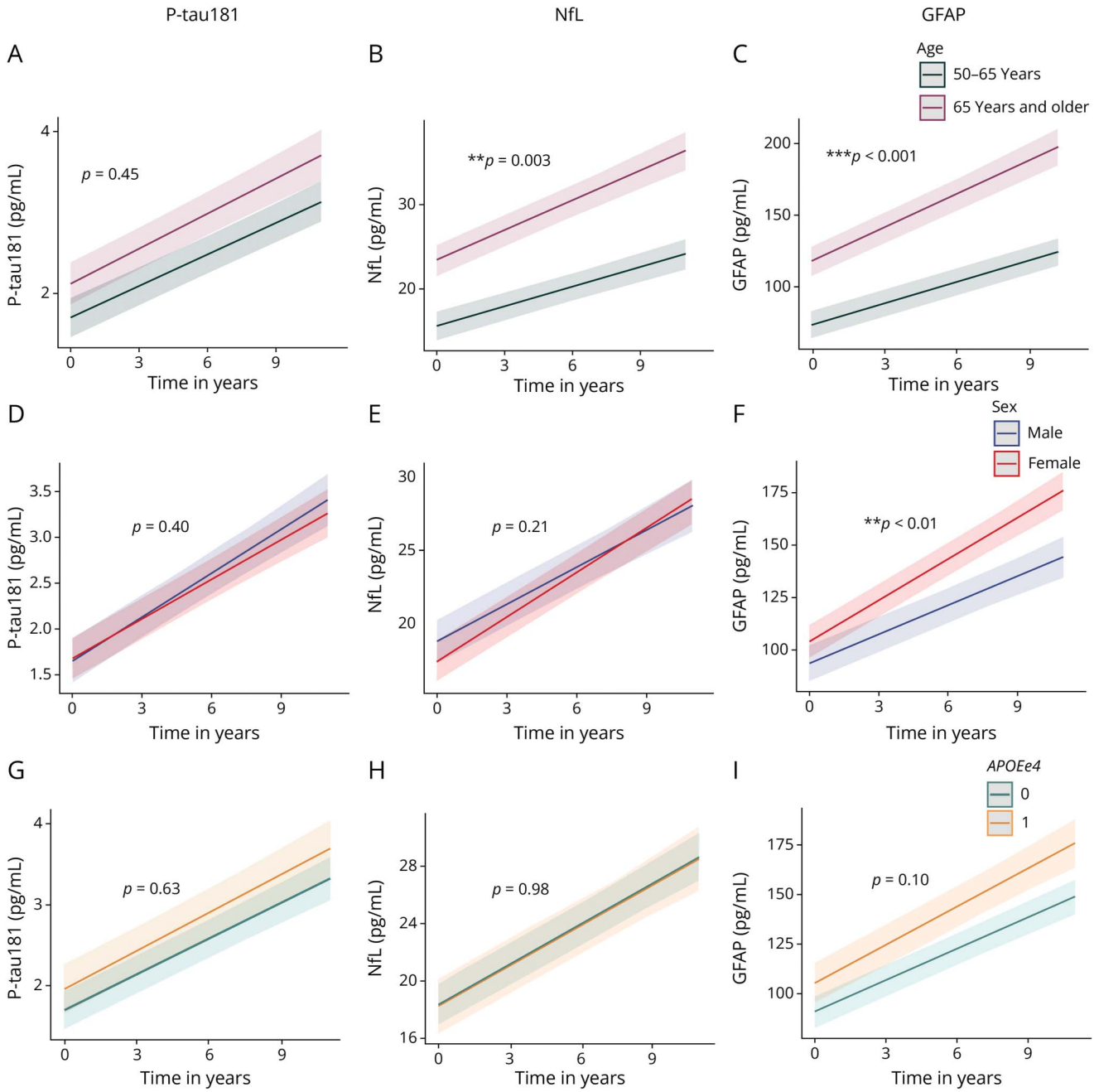
Of interest, the association of age at baseline with P-tau181 and GFAP levels was modified by *APOEe4* status, where *APOEe4* carriers began to experience significantly higher levels of P-tau181 compared with noncarriers between the age of 65 and 70 years and significantly higher levels of GFAP were evident between the age of 60 and 65 years. GFAP levels also began to increase more rapidly during follow-up after only 4 years compared with noncarriers. These results support the idea that GFAP may capture earlier biological processes associated with *APOEe4* status, which could be important in future dementia risk assessment. In addition, this finding may provide information regarding the biological processes involved in early disease pathogenesis, highlighting the role of astrocytic activation in driving further disease processes.

Although the sex specificity of dementia-related blood biomarkers has not yet been extensively explored, previous studies have shown higher NfL CSF levels, but not blood levels, among men[23-25] and higher GFAP blood levels among women.[21,26,27] P-tau181 has not been shown to differ according to sex,[28] which is in line with our study.

Men generally have higher cardiovascular burden compared with women,[29] which could lead to higher rates of NfL as seen in our study. Another possible explanation could be the differences in brain structure according to sex. Men have larger brain volume compared with women,[30] which may translate into increased levels of NfL in blood that do not correspond to increased relative neurodegeneration. It will be important in future research to discern whether the difference in biomarkers based on sex is due to differential dementia risk or rather sex differences in brain structure and function.

Higher GFAP levels and rates of increase were evident in our study in line with several previous studies that have reported higher cross-sectional levels of GFAP among women,[21,26,27] as well as higher rates of change.[9] GFAP is a known marker of AD and dementia risk, and increased astrocytic response may contribute dementia pathogenesis among women.[2,21,31] Sex hormones are believed to influence astrocytic response and neuroinflammation, possibly explaining increased GFAP levels among women. In addition, in secondary analysis, we found higher GFAP levels among women who had not yet gone through menopause, further supporting the connection between sex hormones and GFAP levels. In a recent registry study in Denmark including over 60,000 individuals, a positive

Figure 5 Longitudinal Dementia-Related Biomarker Trajectories by Age, Sex, and *APOEe4* Carriership (A–I)



The x-axis represents time since baseline in years. *p* Values are representative of the interaction between time since baseline and age at baseline, sex, and *APOEe4*, as appropriate (significant differences in the rate of biomarker change). Shaded areas are indicative of 95% confidence intervals. Age analyses (A–C) are adjusted for sex and *APOEe4*; sex analyses (D–F) are adjusted for age and *APOEe4*; and *APOEe4* analyses (G–I) are adjusted for age and sex. *APOEe4*: carrier (1) vs noncarrier (0). GFAP = glial fibrillary acidic protein; NfL =neurofilament light; P-tau181 = phosphorylated tau.

association between estrogen-progesterone therapy and dementia risk was evident.[32] The literature regarding age at menopause and length of reproductive period is quite mixed, with late age at menopause and longer reproductive period lengths showing both positive and negative associations with dementia risk.[33-36] It is, however, possible that neuroinflammation and astrocytic response to sex hormones could be a contributing factor of increased dementia risk among women. In addition, it is possible that sex-dependent responses in the innate and adaptive immune system, where women are known to have a stronger response compared with men, may also contribute to higher GFAP levels among women.[37] Furthermore, stress may affect microglia in a sex-dependent manner, possibly contributing to neuroinflammation.[38] The biological mechanism(s) behind the association of GFAP with female sex should be explored further in future research.

APOEe4 is known to play a paramount role in AD pathogenesis, and several studies have shown higher levels of P-tau181 in blood among *APOEe4* carriers as in our study.[9,39,40] One previous study as in our results found that NfL did not differ by *APOEe4* status[9] while another study of cognitively unimpaired participants found an association between NfL and *APOEe4*.[41] The ESTHER study included participants aged 50–75 years at baseline, and it is possible that changes in NfL were not yet evident. The association of GFAP with *APOEe4* is not clear; in a study of at-risk individuals, *APOEe4* carriers had significantly higher levels of GFAP in line with our results[9] while 2 other studies showed no difference in GFAP levels.[40,42] Our results support the idea that *APOEe4* status may also play a role in astrocytic response. In a molecular study of patient-specific induced pluripotent stem cells, *APOEe4* genotype was shown to induce astrocyte activation leading to neuroinflammation, a factor believed to increase AD risk.[43] Future research should confirm whether *APOEe4* genotype is associated with levels of NfL and GFAP. It is likely that the association between *APOEe4* genotype and dementia-related blood biomarkers is partly dependent on the age of participants studied.

To determine reference values and utilization in risk stratification, it is essential to examine the association of the dementia-related blood biomarkers (P-tau181, NfL, GFAP) with nonmodifiable risk factors such as age and sex. Many established biomarkers have age-specific and sex-specific reference values. Our results indicate that age should be considered for all 3 biomarkers, P-tau181, NfL, and GFAP. Furthermore, sex should be considered in risk stratification/reference value determination for both NfL and GFAP. It is imperative in future research to confirm whether sex-specific differences in the brain account for biomarker differences (e.g., larger brain volume among men) or whether the sex-specific differences in biomarker levels indicate important sex-specific biological processes (e.g., sex hormone–induced astrocyte activation) involved in dementia risk. While *APOEe4* status may not be used for reference value determination, it may be important to consider stratification by *APOEe4* status in risk assessment with biomarkers.

The strengths of this study include the population-based setting of community-dwelling older adults, the very long follow-up period, and the importance of the study aim and findings. The results of this study provide critical information for future utilization of dementia-related blood biomarkers in the community and novel findings regarding sex differences in NfL and GFAP levels.

This study also has some limitations, including the measurement of dementia-related biomarkers at different time points and in lithium-heparin plasma as well as serum and relatively small numbers in stratified analyses. Bridge samples were, however, used to account for biomarker variability, and all samples were standardized using a reference sample normalization method, which is standard practice in such biomarker measurements. GFAP and NfL exhibited very high correlation between lithium-heparin plasma and serum measurements (R = 0.89–0.93); however, P-tau181 exhibited lower correlation between media (R = 0.64), introducing potential bias into follow-up measurements of P-tau181. In addition, in the dementia diagnosis–stratified analyses, there was a possibility of dementia misdiagnosis, underdiagnosis, or delayed diagnosis. The dementia diagnoses in the community-based ESTHER study were clinical diagnoses reported heterogeneously by numerous practitioners, which portrays common practice in the community. The validity of the diagnoses has, however, been supported by previous work, in which the *APOEe4*-AD polygenic risk score distribution among dementia diagnoses closely mirror that in the established literature.[44] Furthermore, the analyses between menopause and biomarker levels did not account for hormone replacement therapy, which may have potentially affected the results. Finally, generalizability is limited to individuals of European descent. It is critical to complete further longitudinal studies in more diverse populations to confirm whether differences in association with nonmodifiable risk factors according to race and ethnicity exist.

In this longitudinal population-based study regarding dementia-related biomarker levels in blood of community-dwelling adults aged 50–75 years at baseline and followed over 17 years, P-tau181 exhibited age and *APOEe4* dependency; NfL, age and sex dependency; and GFAP, age, sex, *APOEe4*, and menopause dependency. GFAP levels and rates of increase were greater in women, in particular among premenopausal participants. Future risk stratification and reference value strategies should consider nonmodifiable determinants of dementia-related biomarkers, and further research should confirm these findings and explore the role of menopause in dementia pathogenesis among women.

Author Contributions

H. Stocker: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. L. Beyer: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. K. Trares: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. J. Stevenson-Hoare: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. D. Rujescu: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. B. Holleczek: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. K. Beyreuther: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. B. Schoettker: drafting/revision of the manuscript for content, including medical writing for content; major role in the

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