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ARTICLE

Mitochondrial DNA abundance in blood is associated with Alzheimer's disease- and dementia-risk

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The mitochondrial cascade hypothesis of Alzheimer's disease (AD) has been portrayed through molecular, cellular, and animal studies; however large epidemiological studies are lacking. This study aimed to explore the association of mitochondrial DNA copy number (mtDNAcn), a marker representative of mtDNA abundance per cell, with risk of incident all-cause dementia, AD, and vascular dementia diagnosis within 17 years and dementia-related blood biomarkers (P-tau181, GFAP, and NfL). Additionally, sex-stratified analyses were completed. In this German population-based cohort study (ESTHER), 9940 participants aged 50–75 years were enrolled by general practitioners and followed for 17 years. Participants were included in this study if information on dementia status and blood-based mtDNAcn measured via real-time polymerase chain reaction were available. In a nested case-control approach, a subsample of participants additionally had measurements of P-tau181, GFAP, and NfL in blood samples taken at baseline. Of 4913 participants eligible for analyses, 386 were diagnosed with incident all-cause dementia, including 130 AD and 143 vascular dementia cases, while 4527 participants remained without dementia diagnosis within 17 years. Participants with low mtDNAcn (lowest 10%) experienced 45% and 65% percent increased risk of incident all-cause dementia and AD after adjusting for age and sex (all-cause dementia: HRadj, 95%CI:1.45, 1.08–1.94; AD: HRadj, 95%CI: 1.65, 1.01–2.68). MtDNAcn was not associated to vascular dementia diagnosis and was more strongly associated with all-cause dementia among women. In the nested case-control study ($n = 790$), mtDNAcn was not significantly associated with the dementia-related blood biomarkers (P-tau181, GFAP, and NfL) levels in blood from baseline before dementia diagnosis. This study provides novel epidemiological evidence connecting mtDNA abundance, measured via mtDNAcn, to incident dementia and AD at the population-based level. Reduced mitochondrial abundance may play a role in pathogenesis, especially among women.

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INTRODUCTION

Dementia affects millions of individuals worldwide and effective disease-modifying treatment and prevention efforts for the most common form of dementia, Alzheimer's disease (AD), remain elusive as the molecular pathogenesis of the disease is inherently complex and has yet to be definitively defined. [1, 2] The amyloid cascade hypothesis of AD asserts Aβ induced tau tangle formation and necroptosis as the basis of AD pathogenesis leading to dementia. [3–5] The mitochondrial cascade hypothesis proposes that altered energy metabolism leads to the pathological consequences associated to AD. [6, 7] It is theorized that Aβ-induced mitochondrial dysfunction and oxidative damage lead to downstream pathological consequences, [6–8] or alternatively that mitochondrial dysfunction initiates AD pathogenesis. [6, 9, 10] Though the definitive role of mitochondria in AD pathogenesis is unclear, it is known that mitochondrial function is critical to neuronal health as the main energy source and for its role in neuronal homeostasis regulation of cell death. [11]

Mitochondrial DNA copy number (mtDNAcn) is a marker representative of mtDNA abundance per cell. [12] Lower mtDNAcn has shown associations to a number of aging-related diseases, all-cause mortality, and has been shown to be evident in the AD brain. [13–17] Higher mtDNAcn has been linked to better current and future cognitive function. [18] Furthermore, the results of a Mendelian randomization analysis have suggested that mtDNAcn play a causative role in dementia risk. [19]

Still, limited research regarding the association between blood-based mtDNAcn and dementia risk exists. One previous study of less than 450 individuals exhibited associations to incident dementia but not AD. [20] While the potential role of mitochondria in AD and dementia has been researched in molecular and animal studies, and a mendelian randomization study has suggested a casual association, [19] a large-scale epidemiological investigation regarding mtDNAcn and incident dementia is lacking. Moreover, the association with blood-based endophenotypes of dementia which have been shown to accurately predict

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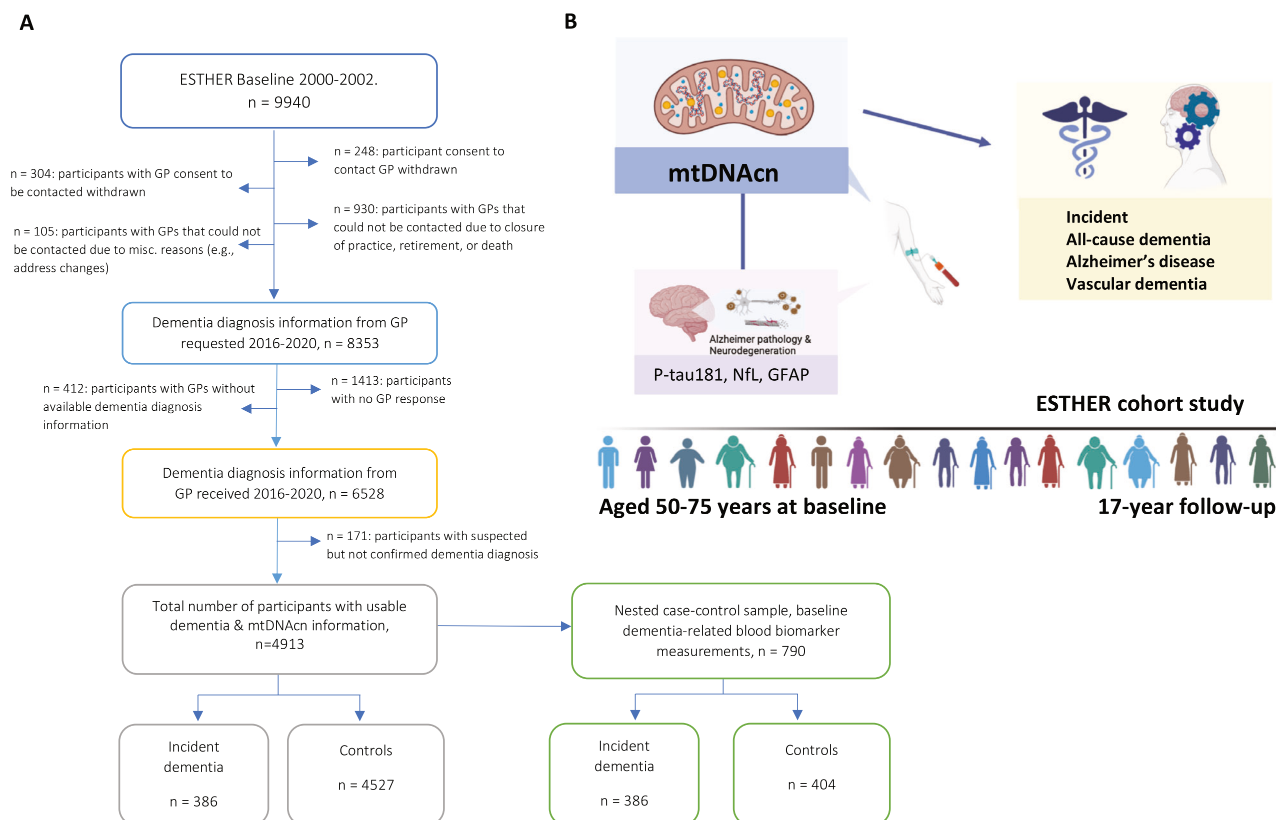

Fig. 1 Flow-chart and schematic representation of the study. A Flow-chart of participants included in the study and (**B**) schematic representation of the study.

amyloid-specific neuropathology, such as phosphorylated tau181 (P-tau181), glial fibrillary acidic protein (GFAP), and neurofilament light (NfL), have not been explored. [21, 22]

Therefore, the aim of this study was to explore the association of blood-based mtDNAcn with risk of incident all-cause dementia, AD, and vascular dementia diagnosis within 17 years and dementia-related blood biomarkers (P-tau181, GFAP, and NfL) in the community-based ESTHER cohort study (Fig. 1). Additionally, mtDNAcn and dementia risk analyses were completed according to sex.

MATERIALS AND METHODS

Study participants and data collection

The ESTHER study is a population-based prospective cohort study of community-dwelling older adults in Germany consisting of 9,940 participants (50–75 years old at baseline) recruited by general practitioners (GPs) in a statewide study in Saarland, Germany in 2000–2002. [23, 24] Participants completed standardized health questionnaires, provided blood and urine samples, and GPs provided medical information. Comprehensive monitoring of major disease incidence and mortality was conducted through participant and GP follow-up 2, 5, 8, 11, 14, and 17 years after recruitment for all participants. Furthermore, data were linked to the Saarland Cancer Registry and death certificates were obtained from local health authorities.

Data regarding dementia diagnoses were collected from participants' general practitioners, who were asked to fill out questionnaires and provide all available medical records regarding dementia diagnoses (or lack of dementia diagnoses) throughout follow-up. [24] The current guidelines in Germany for AD diagnosis follow the National Institute on Aging and the Alzheimer's Association [25] or the International Working group (IWG)-2 criteria, for vascular dementia (VD) diagnosis the National Institute of Neurological Disorders and Stroke (NINDS)- Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) criteria, [26] and all-cause dementia diagnoses are recommended if the dementia symptoms outlined by the ICD-10 are present for at least six months. [27]

The sample for the main analyses included participants with dementia and mtDNAcn information available, n = 4913 (Fig. 1). The secondary analyses including the plasma biomarkers, p-tau181, GFAP, and NfL included all ESTHER participants with dementia, mtDNAcn information, and plasma biomarker measurements, n = 790 (n = 386 all-cause dementia cases; n = 404 controls). The dementia-related blood biomarkers were measured in a nested case control approach including dementia cases and participants without dementia diagnosis throughout follow-up as controls.

Ethics approval and consent to participate

The ESTHER study is an ongoing cohort study conducted in accordance with the Declaration of Helsinki. Informed written consent was collected from all study participants. The ESTHER study was approved by the Ethics Committee of the Medical Faculty at Heidelberg University and the Physicians' Board of Saarland.

MtDNAcn assessment

MtDNAcn measurement was performed in 7640 of the total 9940 ESTHER participants. [12] Real-time multiplex polymerase chain reaction was used to quantify the copy number of the mitochondrial gene NADH dehydrogenase, subunit 1 (ND1), and of the nuclear single copy gene albumin (ALB) as housekeeping reference, using DNA extracted from blood samples. All DNA samples were diluted to a concentration of 2.5 ng/µL in a 96 well plates. The triplicates of each sample were obtained dispensing with an electronic multichannel pipette 2 µL of the sample in 3 wells of a 384-well plate (5 ng DNA of each individual in each well). [28] The polymerase chain reaction (PCR) was performed using a Viia-7 sequence detection system (Applied Biosystems) to acquire the cycle threshold (Ct) values for copy number of ND1 and ALB gene. Samples were excluded if: (1) individual count values that deviated from the average of the triplicates by more than 5% of the standard deviation were excluded (n = 467), (2) the average count was not within the standard curve range (n = 337). Standard curves were generated in each plate with a serial dilution (1:2)

from 30 ng to 0.47 ng of genomic DNA pooled from 50 random individuals belonging to the study. The standard pool (STP) was renewed every 4–5 plates (using however the same subjects), to preserve the quality of the pooled DNA during the laboratory measurement. For each reaction the RT-PCR efficiency (E) was calculated using standard curve points in the exponential phase according to the equation: $E = 10[-1/\text{slope}]$. After exclusion due to quality control (QC) failure, the mtDNAcn was obtained for 6836 individuals as the ratio between ND1/ALB copy numbers, based on the calculation introduced by Pfaffl. [29] This approach is suited to qPCR results lacking identical efficiency between the amplification reaction of ND1 and ALB, using the Ct of the standard curve to the equivalent of 5 ng of DNA as a calibrator.

Dementia-related blood biomarker measurements

Lithium-Heparin and serum samples were stored upon arrival at –80 °C. Prior to analysis on the Simoa™ HD-X Analyzer by Quanterix,™ samples were thawed at room temperature and mixed thoroughly. After a centrifugation step at 10,000 × *g* for 5 min, samples were applied to a conical 96-well plate (Quanterix, USA) and measurements were carried out immediately. For the calculation of concentrations, lot specific calibrators included in the kits were measured as well as one low and one high concentrated lot specific controls. In this study the commercially available Simoa™ Neurology 4-Plex E and 2-plex Advantage Kit (Quanterix, USA) and Simoa™ pTau-181 Advantage V2 Kit (Quanterix, USA) were used according to manufacturer´s instructions and with on-board automated 4x sample dilution. Baseline measurements were completed in heparin samples in two rounds (July 2020 (n = 768) [30] and July 2022 (n = 258)). Bridge samples were utilized to account for between round variability (n = 45 bridge samples). Correlation was first assessed and measurement pairs were excluded if the difference between measurements were greater than two standard deviations (SDs) from the mean difference. Correlation between round one and round two measurements ranged from R = 0.75-0.89. Standardization of round two measurements to round one was completed with ordinal least square regression models.

Measurements that failed quality control during measurement (P-tau181 n = 5, GFAP n = 6, Nfl n = 9) and extreme outliers (> 4 standard deviations from the mean, P-tau181 n = 4, GFAP n = 3, Nfl n = 11) were excluded from analysis.

Covariate data ascertainment at baseline

The following information was ascertained at baseline through self-administered questionnaire and/or physician reports: age, sex, educational level, body mass index (BMI), physical activity level, lifetime history of depression, history of cardiovascular events (myocardial infarction, stroke, death due to cardiovascular disease), any cancer diagnosis, hypertension, diabetes mellitus, smoking status, alcohol use, and fruit and vegetable intake.

Covariate laboratory measurements

Blood samples and urine samples taken at baseline were stored at −80 °C until time of measurement. Serum creatinine measurements were performed by the kinetic Jaffe method. All measurements were performed in a blinded fashion. *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). The estimated glomerular filtration rate test (eGFR) was used to assess kidney function to be used as a covariate in the dementia-related blood biomarker analyses. [31] The eGFR was estimated by the 2021-CKD-EPI creatinine (eGFRcr) equation that does not include race. [32]

Statistical analysis

Baseline characteristics of participants using summary statistics were calculated by dementia diagnosis status. Cumulative incidence curves stratified by mtDNAcn were calculated for all-cause dementia, AD, and VD diagnoses within 17 years in all participants as well as in men and women only.

Cox proportional hazards regression analysis was used to calculate hazard ratios (HRs) including 95% CIs with incident all-cause dementia, AD, and VD diagnoses as the dependent variables and mtDNAcn as the independent variable. End of observation included date of dementia diagnosis, date of death, or date of the 17-year follow-up (date of response from the GP regarding dementia diagnosis status). The proportionality assumption for the Cox models was assessed using the methods outlined by Lin, Wei, and Ying. [33]

Multivariate analysis with various levels of adjustment was conducted to reflect overall predictive ability and independent contribution to the prediction of dementia risk. Model 1 adjusted for age and sex and Model 2 adjusted for age, sex, educational level, *APOE* genotype, body mass index (BMI), physical activity level, lifetime history of depression, history of cardiovascular events (myocardial infarction, stroke, death due to cardiovascular disease), any cancer diagnosis, hypertension, diabetes mellitus, smoking status, alcohol use, and fruit and vegetable intake. There were no missing observations by design for mtDNAcn, dementia diagnoses, age or sex. Multiple imputation (n = 20) for data missing at random was conducted using the Markov chain Monte Carlo method with all included variables and the imputed datasets were utilized for Model 2. A summary of covariate data including missing data is shown in Supplementary Table 1. MtDNAcn was examined as a scaled continuous variable (z-score) and as a binary variable comparing the lowest decile (decile 1) to the upper deciles (decile 2–10). All Cox-regression analyses were additionally completed according to sex, where mtDNAcn deciles for the binary variable were calculated in a sex-specific manner.

The dose-response relationships mtDNAcn and dementia including subtypes was assessed using restricted cubic spline (RCS) functions with four knots at the 5th, 35th, 65th, and 95th percentiles of mtDNAcn.

Cross-sectional analyses were completed in a subsample of ESTHER participants with baseline dementia-related blood biomarker measurements (P-tau181, GFAP, and NfL). Levels of the dementia-related biomarkers by mtDNAcn decile categories were examined. Linear regression analyses were conducted with the plasma biomarkers as the dependent variables and mtDNAcn as the independent variable. The regression analyses were conducted with varying levels of adjustment as outlined above; however all plasma biomarker analyses were adjusted for eGFR as it has been shown to affect biomarker levels. [31] Spearman rank correlation coefficients were calculated to explore the relationship between mtDNAcn and levels of plasma biomarker levels. The dementia-related biomarker analyses were completed in the entire sample and stratified by dementia status.

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 two sided and conducted at an α-level of 0.05. The code utilized for this analysis is not publicly available, but may be made available upon request.

RESULTS

Participant characteristics

This study included 4,913 participants, aged 50–75 years at baseline (mean age: 62 years), and consisted of 55% women (Table 1). Three-hundred and eighty-six participants received an all-cause dementia diagnosis within 17 years including 130 AD diagnoses and 143 VD diagnoses, while 4,527 participants remained without dementia diagnosis throughout follow-up. The mean length of follow-up was 10.9 years in incident dementia cases and 15.1 years in participants that remained without dementia diagnosis. The most common comorbidities of participants were: hypertension (56%), diabetes (15%), and history of depression (15%). Additionally, the mean BMI was 27 kg/m^2 and only a quarter of participants had 10 years or more of formal education (Supplementary Table 1).

Participants diagnosed with all-cause dementia during follow-up had lower mtDNAcn compared to participants that remained without dementia. Unadjusted incidence of all-cause dementia differed in the highest and lowest mtDNAcn deciles: highest mtDNAcn decile (cumulative incidence, incidence-rate: 6.1%, 4.1 cases per 1000 person-years); lowest mtDNAcn decile (cumulative incidence, 10.4%; incidence rate, 7.4 cases per 1000 person-years) (Fig. 2). The incidence of all-cause dementia according to mtDNAcn differed more greatly among women compared to men (cumulative incidence, incidence-rate: Women: highest decile, 6.8%, 3.9 cases per 1000 person-years, lowest decile: 13.5%, 9.4 cases per 1000 person-years; Men: highest decile, 6.8%, 4.8 cases per 1000 person-years, lowest decile, 9.9%., 6.2 cases per 1000 person-years) (Fig. 2).

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Table 1. Summary statistics by dementia diagnosis.

	Participants without dementia diagnosis (0-17 years) 0 (N = 4527)	All-cause dementia (0-17 years) 1 (N = 386)	Alzheimer's disease (0-17years) 1 (N = 130)	Vascular dementia (0-17years) 1 (N = 143)	p value ACD[a]	p value AD[b]	p value VD[c]
Age in years					<0.001	<0.001	<0.001
Mean (SD)	61.29 (6.50)	66.83 (5.20)	67.02 (5.11)	66.67 (5.06)			
Range	50.00–75.00	50.00–75.00	52.00–75.00	52.00–75.00			
Sex					0.449	0.768	0.463
Men	2044 (45.2%)	182 (47.2%)	57 (43.8%)	69 (48.3%)			
Women	2483 (54.8%)	204 (52.8%)	73 (56.2%)	74 (51.7%)			
mtDNAcn					0.067	0.224	0.244
Mean (SD)	1.16 (0.55)	1.10 (0.49)	1.10 (0.41)	1.10 (0.52)			
Range	0.13–7.60	0.17–3.63	0.32 - 2.65	0.17–3.61			
Education					<0.001	0.021	0.029
N-Miss	100 (2.2%)	14 (3.6%)	4 (3.1%)	5 (3.5%w)			
≤9 years	3221 (72.8%)	303 (81.5%)	105 (83.3%)	114 (82.6%)			
10–11years	661 (14.9%)	30 (8.1%)	9 (7.1%)	11 (8.0%)			
>11years	545 (12.3%)	39 (10.5%)	12 (9.5%)	13 (9.4%)			
*APOE*e4 allele					<0.001	<0.001	<0.001
N-Miss	83 (1.8%)	8 (2.1)	2 (1.5%)	4 (2.8%)			
no	3357 (75.5%)	223 (59.0%)	65 (50.8%)	87 (62.6%)			
yes	1087 (24.5%)	155 (41.0%)	63 (49.2%)	52 (37.4%)			

ACD all-cause dementia, *AD* Alzheimer's disease, *SD* standard deviation, *VD* vascular dementia.
[a]p value for comparison between participants diagnosed with all-cause dementia and those that remained without dementia diagnoses within 17 years.
[b]p value for comparison between participants diagnosed with Alzheimer's disease and those that remained without dementia diagnoses within 17 years.
[c]p value for comparison between participants diagnosed with vascular dementia and those that remained without dementia diagnoses within 17 years.

The dementia-related blood biomarker sample included 785 participants, 383 participants with incident dementia diagnosis and 402 participants that remained without dementia diagnosis throughout 17 years. The mean (SD) baseline level of P-tau181 was 1.7 (1.2) pg/mL, GFAP was 101.7 (52.4) pg/mL, and NfL was 17.4 (7.9) pg/mL. The distributions of the dementia-related blood biomarkers are shown in Supplementary Fig. 1.

mtDNAcn and dementia risk

Participants with low mtDNAcn levels (decile 1 vs. decile 2–10) experienced 1.45 times the risk of developing all-cause dementia compared to all other participants after adjustment for age and sex (HR_{adj}, 95%CI:1.45, 1.08–1.94) and significant associations remained after extensive adjustment of possible confounders (Fig. 3). The dose-response restricted cubic spline analysis revealed the greatest increase of risk was seen at the lowest mtDNAcn levels (Supplementary Fig. 2).

Participants with mtDNAcn levels in the lowest decile experienced 1.65 times greater risk of AD diagnosis during follow-up compared to those in all other deciles after adjusting for age and sex (HR_{adj}, 95%CI:1.65, 1.01–2.68) (Fig. 3). MtDNAcn was not associated with an increased risk of vascular dementia diagnosis.

The association of mtDNAcn with dementia risk differed by sex but without evidence of significant interaction (Fig. 3). Women with low mtDNAcn levels experienced 70% greater risk of dementia diagnosis within 17 years, while men did not experience greater risk of dementia based upon mtDNAcn levels after adjusting for age (HR_{adj}, 95%CI: all-cause dementia: women: 1.66, 1.14–2.42; men: 1.32, 0.85–2.07).

mtDNAcn and dementia-related blood biomarkers

The participant characteristics of the dementia-related biomarker sample are presented in Supplementary Table 2. Blood NfL levels were significantly higher in those participants with mtDNAcn levels in the lowest decile compared to the highest decile; however, the dementia-related blood biomarkers were not, or only minimally in the case of NfL, correlated to mtDNAcn (Fig. 4) and in linear regression analyses after adjusting for age and sex there were no significant associations between mtDNAcn and any of the biomarkers (Supplementary Table 2). We have previously shown an association between the dementia-related biomarkers and dementia-risk in ESTHER. [30]

DISCUSSION

In this prospective population-based study (n = 4913), participants with low mtDNAcn at assessment experienced 45% and 65% increased risk of all-cause and AD diagnosis respectively within 17 years. MtDNAcn was more strongly associated with dementia risk among women and lacked associations among men. Finally, in cross-sectional analyses mtDNAcn was not associated to dementia-related blood biomarkers (P-tau181, GFAP, and NfL).

MtDNAcn & dementia risk

Limited research regarding mtDNAcn measured in blood and risk of incident dementia exists. In a study by Yang et al., mtDNAcn was estimated through a combination of whole exome sequencing data and from genotyping arrays in 419 individuals and was significantly associated to incident dementia but not incident AD. [20] In the other study by Chong et al, a mtDNAcn genome-wide association study was performed and subsequent Mendelian randomization analyses portrayed a causal effect between low mtDNAcn and increased dementia risk. [19] Our study provides novel population-based evidence supporting the association of

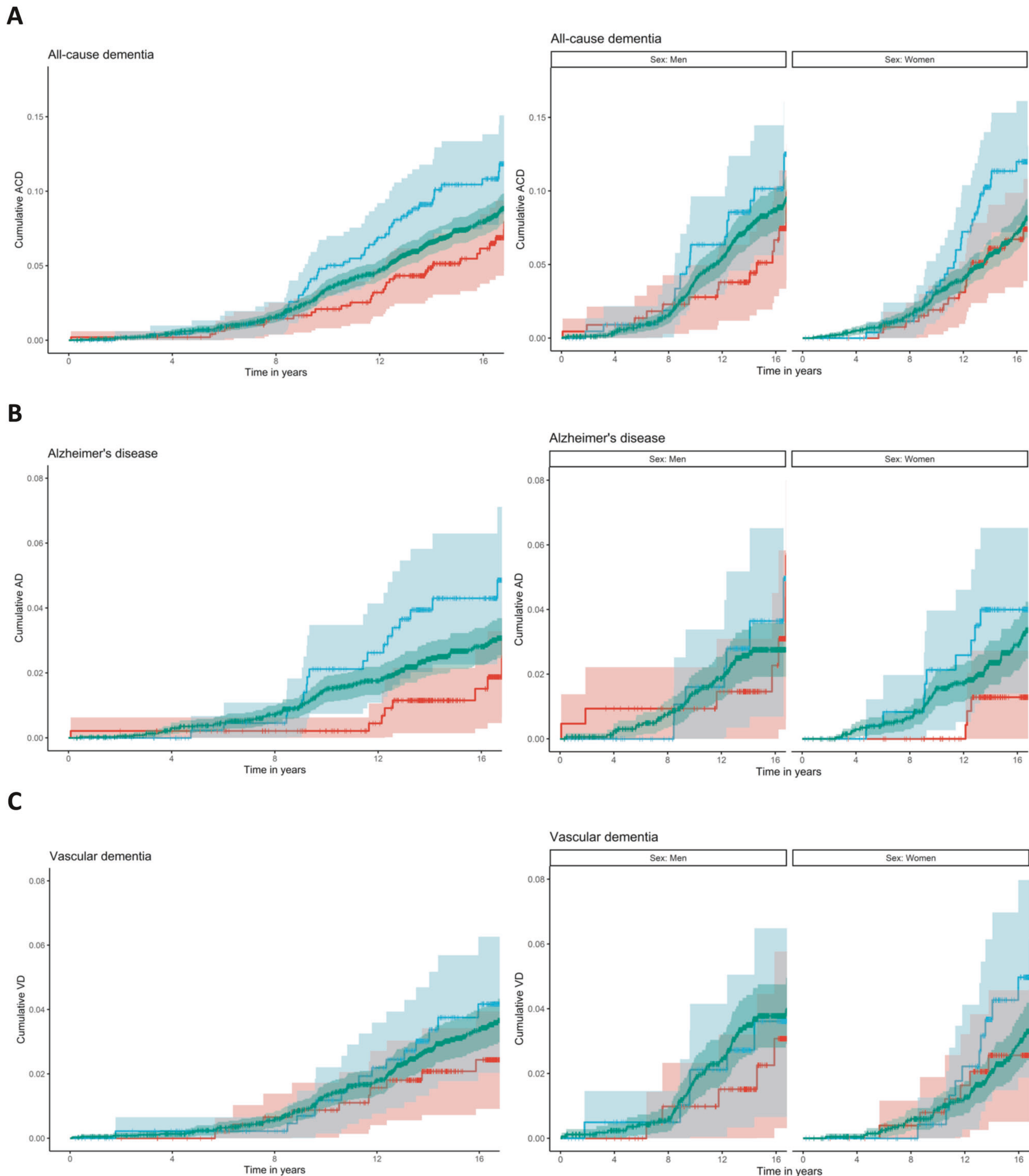

Fig. 2 Cumulative incidence curves of all-cause dementia, Alzheimer's disease, and vascular dementia by mtDNAcn. Cumulative incidence curves of (**A**) all-cause dementia, (**B**) Alzheimer's disease, and (**C**) vascular dementia by mtDNAcn deciles: Levels within the highest decile (red) middle deciles (green), and lowest decile (blue). Number of participants with all-cause dementia (n = 386, decile 10: n = 30, decile 1: n = 50), Alzheimer's disease (n = 130, decile 10: n = 9, decile 1: n = 19), and vascular dementia (n = 143, decile 10: n = 10, decile 1: n = 17). Number of participants without dementia diagnosis throughout follow-up, n = 4527, decile 10: n = 207, decile 1: n = 201.

blood-based mtDNAcn with incident AD, which has yet to be shown among epidemiological studies.

Additionally, it has shown that peripherally measured mtDNAcn abundance was lower among Mexican American participants with cognitive impairment compared to controls [34], and mtDNAcn from post mortem brain tissue has been associated with higher clinical dementia rating and lower cognition. [35] However, the possibility of reverse causation exists in cross-sectional analyses and it is unclear whether mitochondrial function affects cognition and neuropathology or whether increased neurodegeneration in turn decreases mtDNAcn.

The role of mitochondrial function in AD pathology has been widely researched and many molecular, cellular, and animal

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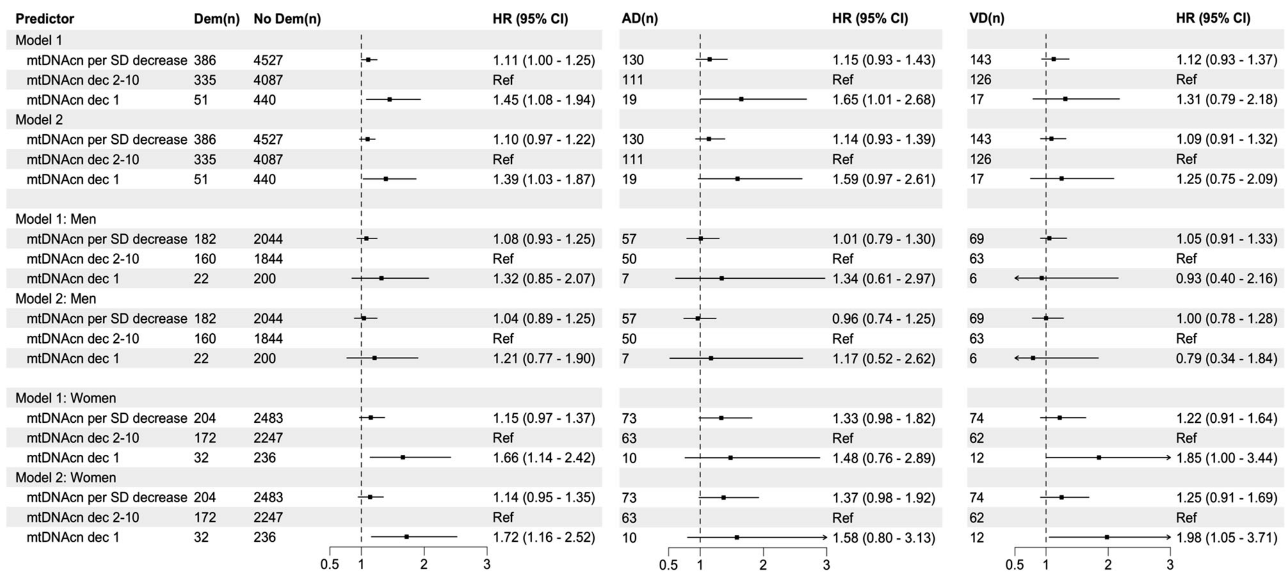

Fig. 3 Cox regression results: association of mtDNAcn with incident diagnosis of all-cause dementia, Alzheimer's disease, and vascular dementia within 17 years. AD Alzheimer's disease diagnosis, Dem All-cause dementia diagnosis, No Dem participants without dementia diagnosis throughout follow-up, VD vascular dementia. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, educational level, *APOE* genotype, body mass index (BMI), physical activity level, lifetime history of depression, history of cardiovascular events (myocardial infarction, stroke, death due to cardiovascular disease), any cancer diagnosis, hypertension, diabetes mellitus, smoking status, alcohol use, and fruit and vegetable intake.

studies have shown a causal link between mitochondrial function and AD and neurodegeneration [7, 36]. The primary mitochondrial cascade hypothesis of AD postulates that mitochondrial dysfunction is the instigator of further AD pathogenesis; while the secondary hypothesis theorizes that Aβ accumulation alters mitochondrial function which then drives further downstream AD physiological processes. [6] Peripheral mitochondrial dysfunction has been found to be altered in AD, suggesting the potential systemic effects involved in disease development and progression. [6] The systemic nature of AD was first suggested more than 35 years ago when important metabolic differences in skin fibroblasts of AD patients compared to controls were found. [37] Our results, which provide novel evidence of the association between mtDNAcn measured in blood and incident dementia and AD many years before diagnosis, further support the importance of systemic processes in AD.

MtDNAcn & dementia risk among women

Previous investigations did not report sex-specific associations between mtDNAcn and incident dementia. In our study, mtDNAcn showed stronger associations to AD than all-cause dementia and was not associated with VD. The association with dementia appeared to be driven by a strong relationship to mtDNAcn among women. In molecular pathology studies it has been shown that estrogen affects mitochondrial function and consequently induces tau deposition and increased oxidative stress. [6, 38, 39] Recently it has been shown that women have greater tau deposition than age-matched men and earlier age at menopause as well as late initiation of hormone therapy particularly increased AD pathology. [40] Mitochondrial dysfunction activated through altering hormone levels could explain increased pathology and risk of dementia diagnosis among women. The role of mitochondria in dementia pathogenesis especially among women should be explored further as a possible explanatory mechanism.

mtDNAcn and dementia-related blood biomarkers

To our knowledge, there has not been any research investigating the relationship between blood-based mtDNAcn and dementia-related biomarkers, but this relationship has been studied in cerebrospinal fluid (CSF). Preclinical patients experienced a decrease in CSF cell-free mtDNA concentration that preceded changes in CSF AD markers, $A\beta_{1-42}$ and p-tau. [41, 42] This was also seen in mutant amyloid precursor protein/presenilin1 (APP/PS1) transgenic mice, where mtDNAcn depleted before synaptic markers altered. [41] In our cross-sectional analyses, we did not see any significant associations between mtDNAcn and dementia-related biomarkers. This could be due to the time-point at which the biomarkers were measured at baseline, many years before most dementia diagnoses where biomarker levels may not have been altered much. However, we have previously shown that the dementia-related biomarkers measured at baseline were also predictive of future dementia diagnosis. [30] mtDNAcn may however capture pathophysiological processes independent of the biomarkers, P-tau181, GFAP, and NfL.

Implications

AD and related dementias have intricate pathogeneses, and the future of dementia etiology identification could conceivably include many different biomarkers which capture distinct processes. The multifaceted nature of dementia requires a multifaceted approach to risk stratification and treatment, and our study provides evidence that mtDNAcn is a promising risk marker of dementia associated to mitochondria. In addition to potential use as a risk-stratification marker, mtDNAcn may provide essential information potential therapeutic targets. For instance, in our study it was revealed that women with low mtDNAcn may be particularly vulnerable to dementia development.

Strengths and weaknesses

The strengths of this study include: the large-scale measurement of mtDNAcn via real time PCR in a uniform manner, the large population-based cohort with a very long follow-up, the extensive epidemiological analyses including many pertinent confounders, and the originality of findings that provide novel epidemiological evidence of the postulated connection between mitochondrial function and incident dementia in particular AD, and the stronger relationship among women.

This study also has some limitations, including the possibility of dementia misdiagnosis, underdiagnosis, or delayed diagnosis. The

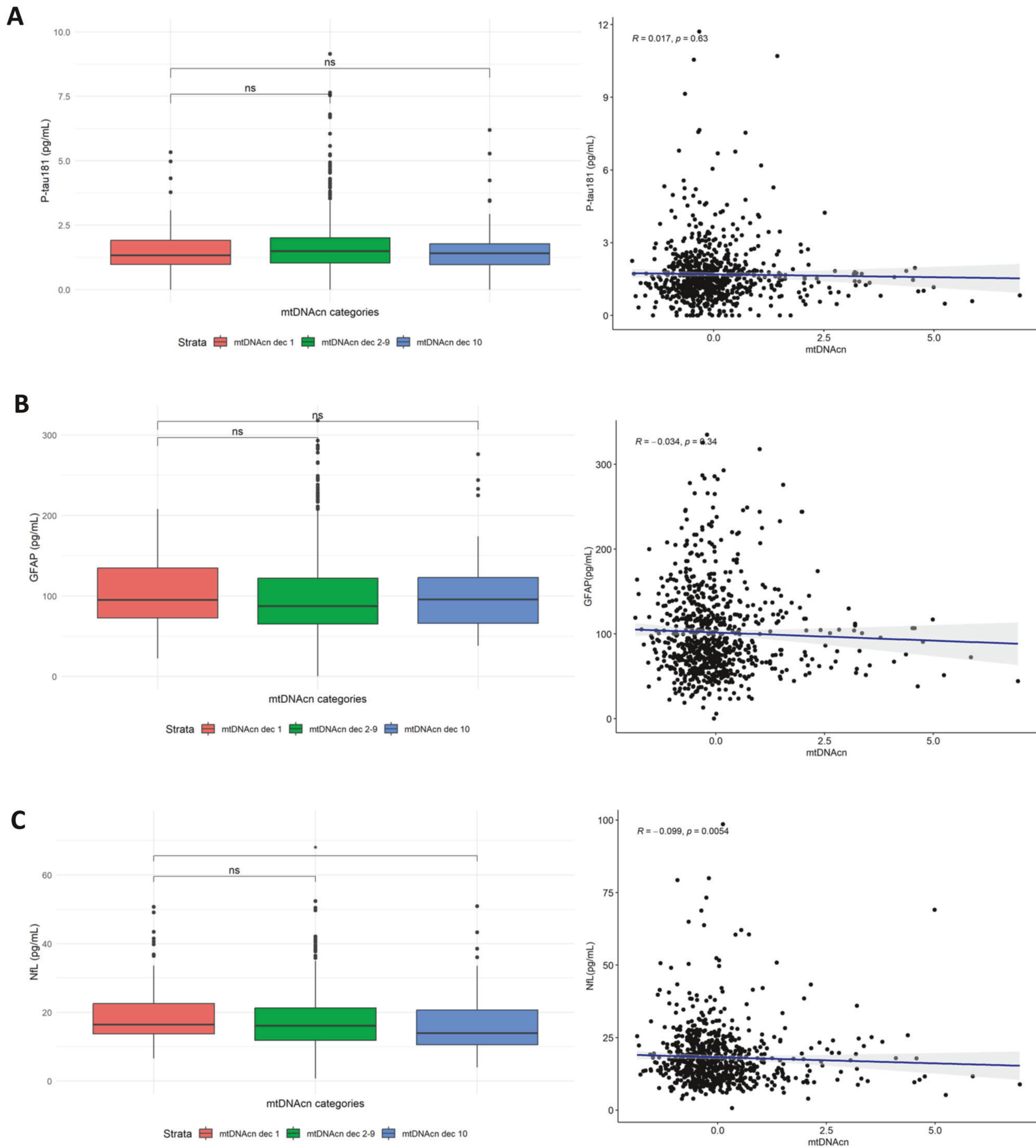

Fig. 4 Dementia-related blood biomarkers by and correlation to mtDNAcn. A P-tau181 (**B**) GFAP (**C**) NfL. *p < .05.

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 *APOE* ε4–AD polygenic risk score distribution among dementia diagnoses closely mirror that in the established literature. [43] Furthermore, blood cell type composition, mitophagy, and loose DNA were not considered in the analyses. Although these factors may have influenced the results, the large sample size and extensive adjustment of potential confounders should have limited bias. The relatively small sample sizes in the sex-stratified and dementia-related blood biomarker analyses may also limit interpretability. Finally, generalizability is limited to individuals of European descent.

CONCLUSION

Low mtDNAcn was associated with increased risk all-cause dementia and AD development but not vascular dementia in a population-based cohort study followed over 17 years. The association with dementia was driven by stronger effects among women. After adjusting for age and sex, mtDNAcn was not

correlated with P-tau181, GFAP, or NfL levels in blood. MtDNAcn in blood could be an important independent biomarker of AD- and dementia-risk which captures early pathophysiological processes associated to mitochondria. Our study provides novel epidemiological evidence regarding mtDNAcn and dementia- and AD risk at the population-level. The sex-specific analyses suggest a potential explanatory mechanism of increased vulnerability among women. Further replication in additional cohort studies is warranted to confirm these findings.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are not publicly available due to local regulations but may be made available from the ESTHER study principal investigator upon reasonable request.

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AUTHOR CONTRIBUTIONS
Dr Stocker had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: HS, HB. Acquisition, analysis, or interpretation of data: HS, MG, LB, KT, LP, DR, BH, KG, BS, DC, FC, HB. Drafting of the manuscript: HS. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: HS. Obtained funding: HB. Administrative, technical, or material support: MG, HS, LB, KG, BS, HB. Supervision: KB, DC, KG, HB.

COMPETING INTERESTS
The authors declare no competing interests.

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