Factors related to blood-based biomarkers for neurodegenerative diseases and their intergenerational associations in the Young Finns Study: a cohort study

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Summary

Background Blood-based biomarkers (BBM) of neurodegenerative diseases are emerging as cost-effective tools in the differential diagnostics of Alzheimer's disease and other dementias. Scarce data exist about factors explaining BBM variation in population-based cohorts, and their intergenerational associations are unknown. This study aimed to characterise BBM distributions among a population-based cohort, investigate the association of a wide array of factors with BBM both in midlife and old age, and investigate intergenerational associations of BBM.

Methods We measured BBM detecting amyloid β and tau pathologies, including amyloid β42, amyloid β40, and phosphorylated Tau (pTau)-217, as well as glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL) in the multigenerational Young Finns Study participants (n=1237, age 41–56 years) and their parents (n=814, age 59–90 years) using the Quanterix Simoa HD-X analyser. Standard statistical methods were used to examine the associations between BBM and age, sex, genetic factors, a plethora of cardiometabolic markers, liver and kidney function, and lifestyle factors, as well as their intergenerational associations.

Findings Increased age was associated with adverse BBM concentrations. Of the various investigated factors, the most robust associations towards adverse BBM concentration were found for *APOE* ε4 carrier status among parents (amyloid β42:40 ratio, pTau-217, and GFAP) and high serum creatinine concentration in both generations (pTau-217, GFAP, and NfL). Several factors related to glucose metabolism and dyslipidaemia were negatively associated with all BBM, but adjusting for BMI diluted many of these associations. Statistically significant intergenerational correlations ranged from 0·20 to 0·33 and were mostly observed between mothers and offspring in pTau-217, GFAP, and NfL. No intergenerational correlations existed in amyloid β42:40 ratio.

Interpretation We identified several factors that might influence BBM concentrations, parental transmission being one of them. For reliable use of BBM in clinical practice, it is important to identify which factors directly link to amyloid β and tau pathology and which factors influence BBM concentrations due to other physiological processes.

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Introduction

Blood-based biomarkers (BBM) are emerging as a cost-effective and minimally invasive method to detect Alzheimer's disease and related neurodegenerative diseases at an early stage of the disease continuum.[1–3] In current clinical practice, Alzheimer's disease is diagnosed using expensive PET and invasive cerebrospinal fluid samples, which limit their usage especially at the early stage of dementias and in low-income countries.[4–6] Developments in ultrasensitive blood-based technologies enable the detection of very low concentrations of BBM obtained from a venous blood sample. According to the 2024 guidelines by the Alzheimer's Association workgroup, core Alzheimer's disease biomarkers involving BBM that can detect amyloid β and tau pathologies could be sufficient to diagnose Alzheimer's disease once cutpoints have been established.[7] Decreased amyloid β42:40 ratio is validated to detect amyloid β pathology but its clinical usability might be limited by a small difference between individuals who are amyloid β positive and amyloid β negative assessed by PET.[3,8–10] Among

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For the Finnish translation of the abstract see Online for appendix 2

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Research in context

Evidence before this study

In 2024, the Alzheimer's Association workgroup released updated research guidelines for the diagnosis and staging of Alzheimer's disease. The new guidelines suggest that blood-based biomarkers (BBM) detecting amyloid β and tau pathologies could be sufficient to diagnose Alzheimer's disease. However, standardised cutpoints for BBM are yet to be determined because studies with diverse populations along the Alzheimer's disease continuum are scarce. We searched PubMed for articles published in English between Nov 4, 2014 and Nov 4, 2024 related to the factors associated with BBM. We used the search terms "blood-based biomarkers", "plasma biomarkers", "amyloid", "tau", "Alzheimer's disease", and "cognitive decline". Previous studies done mostly on older participants with mild cognitive impairment or Alzheimer's disease suggest that several factors such as age, *APOE* ε4 genotype, and certain medical conditions could influence BBM concentrations. In addition, family history is one of the strongest risk factors for late-onset Alzheimer's disease, and previous studies suggest that maternal history of Alzheimer's disease might increase the risk more than that of paternal history of the disease.

Added value of this study

Our observations on wide BBM distributions and high concentrations even among participants in middle age suggest caution when interpreting a single abnormal BBM measure. Our results provide further evidence on several determinants for BBM beginning from midlife. Additionally, our study was the first to reveal that parental, especially maternal, transmission could be an important contributor to offspring's BBM concentrations. The current observations could be applied to the establishment of cutpoints for diagnostic purposes and to generate hypotheses for further studies focusing on revealing the biological pathways behind the associations.

Implications of all the available evidence

Available evidence suggests that BBM cutpoints should be established age-specifically defining not only normal and pathological values but also intermediate zones, in which several samplings should be considered before clinical decision making. The evidence on the factors exerting their influence on BBM and our novel observation on intergenerational transmission of BBM could be applied when interpreting the BBM concentrations in clinical practice, and when considering screening of at-risk individuals with a family history of Alzheimer's disease.

different phosphorylated tau variants, phosphorylated tau 217 (pTau-217) is the most promising BBM for clinical implementation because of its high accuracy in detecting early Alzheimer's disease pathology and predicting future cognitive decline.[11–16] Besides these core Alzheimer's disease biomarkers, non-specific BBM (eg, glial fibrillary acidic protein [GFAP] and neurofilament light chain [NfL]) can complement Alzheimer's disease staging and monitoring.[7] GFAP is mainly expressed in brain astrocytes and is increased in individuals with early amyloid β pathology but also in other neurodegenerative diseases.[17–19] NfL is a marker of axonal injury, and it is increased in a wide variety of neurological conditions as a consequence of axonal damage.[17,20–22]

Before BBM can be used in clinical practice, it is important to understand the associations of comorbidities and lifestyle factors with BBM. Previous studies done mostly in older populations at varying stages along the Alzheimer's disease continuum have indicated that *APOE* ε4 carrier status, renal dysfunction, dyslipidaemia, type 2 diabetes, obesity, and BMI can influence BBM concentrations.[23–28] Moreover, family history is one of the strongest risk factors for late-onset Alzheimer's disease,[29–31] and maternal history of Alzheimer's disease can increase an offspring's risk more than that of paternal history of disease.[32–35] However, given a similar family history, Alzheimer's disease risk might be sex specific.[29] Heritability of BBM is scarcely studied. A twin study among men aged 60–73 years showed that except for amyloid β42:40 ratio, BBMs were heritable.[36] It remains unclear whether BBM concentrations are associated across generations and whether these associations are sex specific.

This substudy of the multigenerational Cardiovascular Risk in Young Finns Study (YFS) includes Finnish participants in two generations, comprising individuals in middle age and their parents. This study aimed to characterise BBM distributions among a population-based cohort, to investigate the association of a wide array of factors, including *APOE*ε4 status, a plethora of cardiometabolic markers, liver and kidney function, and lifestyle factors with BBM both in midlife and old age, and to investigate intergenerational associations of BBM.

Methods

Study population

The national multicentre YFS, started in 1980, was originally designed to provide evidence on the role of early-life genetic and environmental exposures for cardiovascular diseases.[37] During the latest follow-up in 2018–20, the study was extended to the parents and children of the original YFS participants. We contacted the original YFS participants (generation G1) to inform them about the beginning of the new multigenerational data collection. G1 participants were able to opt out of their parents (generation G0) and offspring (generation G2; not included in this substudy) being contacted. For G1 participants who agreed to family involvement, we asked about their parents' severe movement disability and dementia diagnosis, which were considered

as exclusion criteria, and these individuals were not invited to the study. No formal clinical assessment of cognitive function was done.

This study was done in line with the principles of the Declaration of Helsinki. Ethical committees of the Hospital District of Southwest Finland and the European Research Council approved the study. All participants signed written informed consent. The participants had the right to refuse any part of the study protocol or discontinue at any time without the need to provide any explanation.

BBMs

BBM concentrations were measured from fasting blood samples that were collected by venipuncture in EDTA tubes. Samples were centrifuged at room temperature at 2000 g for 10 min and plasma samples were stored at –80°C in the Turku University Hospital. Amyloid β40, amyloid β42, GFAP, and NfL concentrations were measured in Turku University Hospital Clinical Chemistry Laboratory (Turku, Finland) in August, 2023. Quantification was done using a Single molecule array (Simoa) HD-X Analyzer (Quanterix, Billerica, MA, USA) using a Neurology 4-Plex E kit (103670; Quanterix) to simultaneously quantify amyloid β40, amyloid β42, NfL, and GFAP concentrations, and amyloid β42:40 ratio was calculated. Six random samples from both generations were analysed twice to determine within-run variation coefficients which were less than 10% for all assays.

For quantification of pTau-217, frozen plasma samples were sent to the Clinical Neurochemistry Laboratory (Sahlgrenska University Hospital, Gothenburg, Sweden) and quantified using Simoa HD-X Analyzer (Quanterix) and a p-Tau 217 (Simoa ALZpath) assay (Quanterix). Two quality-control concentrations (0·4 pg/mL and 1·7 pg/mL) were run in duplicates at the beginning and at the end of each run. Within-run variation was 5·4% for 0·4 pg/mL and 5·5% for 1·7 pg/mL.

For amyloid β42:40 ratio, lower value indicates more neurodegeneration, whereas for pTau-217, GFAP, and NfL, higher concentration indicates more neurodegeneration.[1]

Genetics, cardiometabolic markers, liver and kidney function, other diseases, and lifestyle factors

We studied a wide array of factors that might potentially associate with BBM concentrations (appendix 1 pp 2–3), including genetics (*APOE* ε4 allele carrier status and polygenic risk score [PRS] for Alzheimer's disease, the APOE single nucleotide polymorphisms [SNPs] rs429358 and rs7412 and SNPs correlating with the two APOE SNPs were removed), vascular diseases and dyslipidaemia (vascular disease, hypertension, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and lipoproteins), glucose metabolism (fasting glucose, fasting insulin, haemoglobin A_{1c}, and type 2 diabetes), liver and kidney function (fatty liver, alanine aminotransferase, aspartate aminotransferase, and serum creatinine), other diseases and disorders (cancer, migraine, depression, and anxiety), and lifestyle factors (education, physical activity index, diet score, smoking, and alcohol consumption).

Statistical analysis

The distributions of the variables were evaluated by visual inspection using quantile–quantile plots and histograms. pTau-217, GFAP, and NfL were skewed, and logarithmic transformation was done. A few extremely high BBM concentrations were identified by visual inspection for all BBM (appendix 1 p 5) and the characteristics of these individuals described (appendix 1 p 6). Given that extremely high BBM concentrations were observed in every individual only in one BBM, we considered these extreme values as sporadic outliers because of technical or unknown reasons and removed these extreme values from the dataset. In addition, a few values below the detection limit in amyloid β40 and amyloid β42 were removed. The difference in BBM by generation (G1 and G0) was examined using *t* tests. Spearman correlations between BBM were calculated using untransformed data.

The association between age, sex, and BMI with BBM was studied using a linear model applied to the whole study population. Then, associations of different factors with BBM were evaluated. As our preliminary analyses suggested that many of the evaluated factors had statistically significant interaction with age, generation-specific age and sex-adjusted linear regression analyses were done separately for each factor (model 1). Because BMI or increased blood volume due to obesity for instance could be potential confounders,[23–25] we repeated the analyses adjusting additionally for BMI (model 2). Effect sizes from both models are visualised in forest plots and numerical results are presented in the appendix 1 (pp 10–14). BBM were converted to Z scores using generation-specific means and SDs for comparability. To compare the effect sizes of individual variables with BBM, each variable was dichotomised (0 indicating the reference, no diseases, laboratory values within reference, and a healthy lifestyle, and 1 indicating a disease, laboratory values outside reference values, and an unhealthy lifestyle). Results obtained using continuous variables are provided in the appendix 1 (p 15). For Alzheimer's disease PRS, genetic principal components (C1–10) were calculated from directly genotyped genetic variants and were additionally included into the models 1 and 2 to account for population stratification. In addition, chip variable was used to account for bias caused by two different genotyping array types in G1 participants.

See Online for appendix 1

Intergenerational associations between BBM were visualised in scatterplots and investigated by calculating sex-specific partial Pearson's correlations controlling for both parent's and offspring's age. To evaluate the effect size of parent's BBM on offspring's BBM, several linear models were constructed, in which the dependent variable was offspring's BBM and explanatory variables were parent's corresponding BBM, offspring's age, BMI, and the factors that were identified to potentially associate with a given BBM in the G1 generation in the previous analyses. All the

explanatory variables were entered into the model simultaneously, and stepwise elimination of non-significant variables was done. Separate models were constructed for each BBM and family relation (daughters and mothers, daughters and fathers, sons and mothers, and sons and fathers).

Statistical analyses were done using R (version 4.2.1), and the level of statistical significance was set at $p \leq 0{\cdot}05$.

Role of the funding sources

The funders of this study had no role in study design, data collection, data analysis, data interpretation, or writing of this report.

Results

2051 Finnish (White) participants with BBM measures were included, of whom 1237 were in middle age (G1 generation, aged 41–56 years) and 814 were their parents (G0 generation, aged 59–90 years) (table 1, appendix 1 pp 4, 7). The amyloid β42:40 ratio was lower and pTau-217, GFAP, and NfL concentrations were higher in G0 participants, indicating a higher amount of neurodegeneration in old age (figure 1). Notably, the BBM distributions were wide in both generations and also some of the G1 participants had high BBM concentrations. BBM concentrations stratified by sex and age groups in 10-year intervals are reported in the appendix 1 (p 8). All correlations between BBMs were statistically significant (appendix 1 p 9).

We first examined the effects of age, sex, and BMI on BBM in the whole study population (table 2). Age was associated with higher pTau-217, GFAP, and NfL and lower amyloid β42:40 ratio with effect sizes ranging from 0·29 to 0·60 SD towards more neurodegeneration for 10 years of age. Male sex was associated with lower amyloid β42:40 ratio and GFAP and higher pTau-217. Higher BMI was associated with lower concentrations in all BBM. The R^2 values of the models were around 0·60 for GFAP and NfL but lower for core Alzheimer's disease biomarkers amyloid β42:40 and pTau-217. Age contributed more than sex and BMI to the goodness of fit in all BBM (table 2).

Age-adjusted and sex-adjusted associations of different factors with BBM (model 1) are shown in figure 2A, and adjusted for age, sex, and BMI (model 2) in figure 2B (numerical results in the appendix 1 pp 10–14). *APOE* ε4 carrier status was associated with adverse amyloid β42:40 ratio, pTau-217, and GFAP concentrations in G0 participants and with adverse amyloid β42:40 ratio in G1 participants and these associations persisted after adjusting additionally for BMI (figure 2). Similarly, Alzheimer's disease PRS was adversely associated with BBMs in the G0 generation but not the G1 generation, with and without additional adjustment for BMI.

Vascular diseases were associated with adverse pTau-217 and NfL concentrations in G0 participants but not in G1 participants. Variables related to dyslipidaemia and glucose metabolism (eg, hypertension, HDL, triglycerides, fasting glucose, insulin, and HbA_{1c} outside reference

	G0 (n=814)	G1 (n=1237)	Overall (n=2051)
Age, years	73·2 (5·46)	49·3 (4·99)	58·8 (12·8)
Sex			
Female	504 (61·9%)	715 (57·8%)	1219 (59·4%)
Male	310 (38·1%)	522 (42·2%)	832 (40·6%)
APOE ε4 carrier status			
Non-carrier	501 (61·5%)	766 (61·9%)	1267 (61·8%)
Carrier	228 (28·0%)	446 (36·1%)	674 (32·9%)
One allele	209 (25·7%)	394 (31·9%)	603 (29·4%)
Two alleles	19 (2·3%)	52 (4·2%)	71 (3·5%)
Missing data	85 (10·4%)	25 (2·0%)	110 (5·4%)
Vascular disease*			
No	486 (59·7%)	1073 (86·7%)	1559 (76·0%)
Yes	311 (38·2%)	137 (11·1%)	448 (21·8%)
Missing data	18 (2·2%)	27 (2·2%)	45 (2·2%)
Hypertension†			
No	175 (21·5%)	760 (61·4%)	935 (45·6%)
Yes	637 (78·3%)	476 (38·5%)	1113 (54·3%)
Missing data	2 (0·2%)	1 (0·1%)	3 (0·1%)
Total cholesterol			
Within range	438 (53·8%)	556 (44·9%)	994 (48·5%)
≥5·0 mmol/L	376 (46·2%)	681 (55·1%)	1057 (51·5%)
HDL cholesterol			
Within range	574 (70·5%)	863 (69·8%)	1437 (70·1%)
Female <1·2 mmol/L, male <1·0 mmol/L	240 (29·5%)	374 (30·2%)	614 (29·9%)
LDL cholesterol			
Within range	441 (54·2%)	486 (39·3%)	927 (45·2%)
≥3·0 mmol/L	360 (44·2%)	714 (57·7%)	1074 (52·4%)
Missing data	13 (1·6%)	37 (3·0%)	50 (2·4%)
Triglycerides			
Within range	603 (74·1%)	954 (77·1%)	1557 (75·9%)
≥1·7 mmol/L	211 (25·9%)	283 (22·9%)	494 (24·1%)
Lipoprotein a			
Within range	582 (71·5%)	908 (73·4%)	1490 (72·6%)
≥300 mg/L	185 (22·7%)	251 (20·3%)	436 (21·3%)
Missing data	47 (5·8%)	78 (6·3%)	125 (6·1%)
Fasting glucose			
Within range	578 (71·0%)	1064 (86·0%)	1642 (80·1%)
≥6·0 mmol/L	236 (29·0%)	173 (14·0%)	409 (19·9%)
Fasting insulin			
Within range	748 (91·9%)	1123 (90·8%)	1871 (91·2%)
≥20·0 mU/L	65 (8·0%)	110 (8·9%)	175 (8·5%)
Missing data	1 (0·1%)	4 (0·3%)	5 (0·2%)
Haemoglobin A_{1c}			
Within range	406 (49·9%)	1019 (82·4%)	1425 (69·5%)
≥42·0 mmol/mol	407 (50·0%)	217 (17·5%)	624 (30·4%)
Missing data	1 (0·1%)	1 (0·1%)	2 (0·1%)
Type 2 diabetes			
No	653 (80·2%)	1153 (93·2%)	1806 (88·1%)
Yes	132 (16·2%)	53 (4·3%)	185 (9·0%)
Missing data	29 (3·6%)	31 (2·5%)	60 (2·9%)
Fatty liver			
No	592 (72·7%)	879 (71·1%)	1471 (71·7%)
Yes	220 (27·0%)	357 (28·9%)	577 (28·1%)
Missing data	2 (0·2%)	1 (0·1%)	3 (0·1%)

(Table 1 continues on next page)

	G0 (n=814)	G1 (n=1237)	Overall (n=2051)
(Continued from previous page)			
ALAT			
Within range	759 (93·2%)	1073 (86·7%)	1832 (89·3%)
Female ≥35 U/L, male ≥50 U/L	55 (6·8%)	164 (13·3%)	219 (10·7%)
ASAT			
Within range	745 (91·5%)	1082 (87·5%)	1827 (89·1%)
Female ≥35 U/L, male ≥45 U/L	68 (8·4%)	153 (12·4%)	221 (10·8%)
Missing	1 (0·1%)	2 (0·2%)	3 (0·1%)
Creatinine			
Within range	672 (82·6%)	1124 (90·9%)	1796 (87·6%)
Female ≥90 µmol/L, male ≥100 µmol/L	142 (17·4%)	113 (9·1%)	255 (12·4%)
Cancer			
No	694 (85·3%)	1179 (95·3%)	1873 (91·3%)
Yes	93 (11·4%)	28 (2·3%)	121 (5·9%)
Missing data	27 (3·3%)	30 (2·4%)	57 (2·8%)
Migraine			
No	722 (88·7%)	1014 (82·0%)	1736 (84·6%)
Yes	66 (8·1%)	191 (15·4%)	257 (12·5%)
Missing data	26 (3·2%)	32 (2·6%)	58 (2·8%)
Depression			
No	737 (90·5%)	1097 (88·7%)	1834 (89·4%)
Yes	47 (5·8%)	110 (8·9%)	157 (7·7%)
Missing data	30 (3·7%)	30 (2·4%)	60 (2·9%)
Anxiety			
No	761 (93·5%)	1129 (91·3%)	1890 (92·2%)
Yes	23 (2·8%)	74 (6·0%)	97 (4·7%)
Missing data	30 (3·7%)	34 (2·7%)	64 (3·1%)
Education‡			
Higher than median	365 (44·8%)	599 (48·4%)	964 (47·0%)
Lower than median	365 (44·8%)	599 (48·4%)	964 (47·0%)
Missing data	84 (10·3%)	39 (3·2%)	123 (6·0%)
Physical activity index‡			
Higher than median	373 (45·8%)	584 (47·2%)	957 (46·7%)
Lower than median	372 (45·7%)	583 (47·1%)	955 (46·6%)
Missing data	69 (8·5%)	70 (5·7%)	139 (6·8%)
Diet score‡			
Higher than median	395 (48·5%)	593 (47·9%)	988 (48·2%)
Lower than median	394 (48·4%)	592 (47·9%)	986 (48·1%)
Missing data	25 (3·1%)	52 (4·2%)	77 (3·8%)
Smoking			
Never, ceased, or on a break	718 (88·2%)	1000 (80·8%)	1718 (83·8%)
At least sometimes	43 (5·3%)	201 (16·2%)	244 (11·9%)
Missing data	53 (6·5%)	36 (2·9%)	89 (4·3%)
Alcohol			
Never or seldom	424 (52·1%)	321 (25·9%)	745 (36·3%)
At least once a month	372 (45·7%)	893 (72·2%)	1265 (61·7%)
Missing data	18 (2·2%)	23 (1·9%)	41 (2·0%)

(Table 1 continues in next column)

(Continued from previous column)

Data are mean (SD) or n (%). ALAT=alanine aminotransferase. ASAT=aspartate aminotransferase. HDL=high-density lipoprotein. LDL=low-density lipoprotein. *Participant has received at least one of the following diagnoses: cardiac infarction, coronary heart disease, heart failure, atrial fibrillation, other arrhythmia, valvular defect, dilation of aorta, constriction of carotid artery, claudication, stroke, or cerebral haemorrhage. †Systolic blood pressure ≥140 mm Hg with or without diastolic pressure ≥90 mm Hg and with or without antihypertensive medication. ‡The variable was dichotomised by calculating generation-specific medians as the variable does not have a recommended cutoff value.

Table 1: **Participant characteristics among parents (G0), offspring (G1), and the overall population**

values, and type 2 diabetes) were associated with favourable concentrations, especially in GFAP and NfL. However, these variables were associated with adverse amyloid β42:40 ratio mostly among G1 participants. After adjusting for BMI, most of these associations were attenuated among G1 participants, whereas associations remained in G0 participants, especially for GFAP. Serum HDL cholesterol outside reference values (females <1·2 mmol/L, males <1·0 mmol/L) were associated with adverse amyloid β42:40 ratios in both generations.

Liver and kidney function had no association with amyloid β42:40 ratio. For the other BBMs, fatty liver and high alanine aminotransferase concentration were associated with lower BBM concentrations in both generations, but these associations were not significant in the G1 generation after adjusting additionally for BMI. High creatinine concentrations were associated with adverse pTau-217, GFAP, and NfL in both generations, and adjustment for BMI did not affect the results.

Anxiety was associated with adverse NfL in the G1 generation and depression with favourable amyloid β42:40 ratio and pTau-217 in the G0 generation. Cancer and migraine were not associated with any of the BBMs in either generation.

Standardised effect sizes for education, physical activity index, and diet score were modest and were further reduced after adjusting for BMI. Smoking was associated with favourable GFAP and alcohol usage with favourable pTau-217 even after adjusting for BMI in both generations.

As a sensitivity analysis, we repeated the analysis using continuous variables when feasible (appendix 1 p 15). The results resembled those obtained using dichotomised variables (figure 2).

After adjusting for BMI, *APOE* ε4 carrier status and serum creatinine concentrations remained the strongest factors associated with adverse BBM. The number of ε4 alleles had an effect on BBM distribution (figure 3). In G0, participants with one or two ε4 alleles had a lower amyloid β42:40 ratio and higher pTau-217 concentration than non-carriers, indicating more amyloid β pathology. However, in G1, only individuals with two ε4 alleles had a lower amyloid β42:40 ratio than those with zero or one ε4 allele, whereas other BBMs were similar regardless of *APOE* ε4 carrier status (figure 3A). Individuals with serum creatinine outside the reference value (females

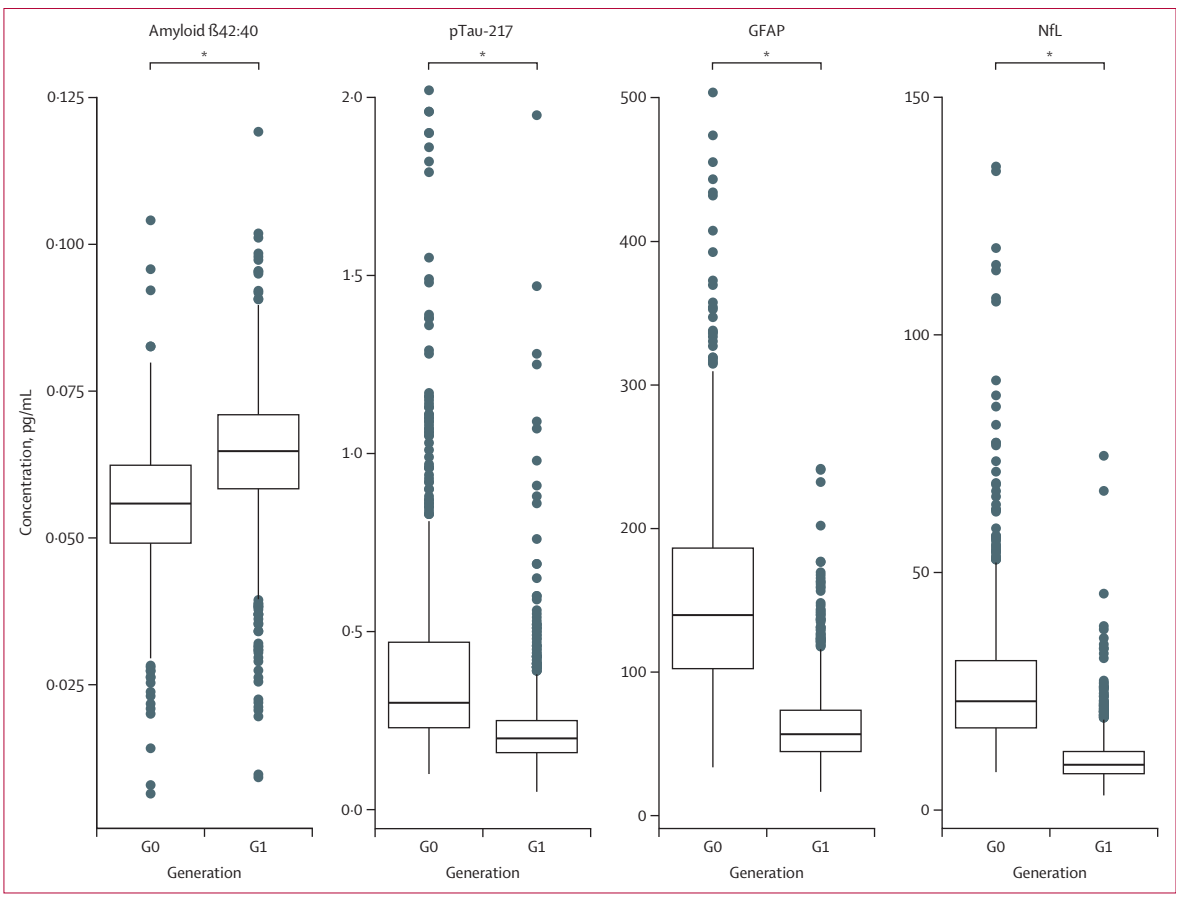


Figure 1: **Distribution of BBM within generations G0 and G1**
Boxplots are based on untransformed data, but the difference between generations is tested using logarithmic transformations for skewed BBMs (pTau217, GFAP, and NfL). *p<0·001 for the difference between the generations. BBM=blood-based biomarkers. GFAP=glial fibrillary acidic protein. NfL=neurofilament light chain. pTau217=phosphorylated Tau 217.

≥90 µmol/L, males ≥100 µmol/L) had higher pTau-217, GFAP, and NfL concentrations, especially among G0 participants (figure 3B).

We visually assessed intergenerational associations of BBM (appendix 1 p 16), and we calculated sex-specific partial correlations controlling for both the age of the parents and offspring. No statistically significant correlations were observed in the amyloid β42:40 ratio. For the other BBMs, statistically significant correlations ranged from 0·21 (95% CI 0·08–0·34 for mothers and sons and p-Tau-217 concentrations) to 0·35 (0·23–0·46 for mothers and sons and Nfl concentrations) and were mostly observed between mothers and offspring (table 3). As a sensitivity analysis, we calculated similar partial correlations by including only G1 participants who had BBM measures from both parents (n=134 for G1 female participants and n=109 for G1 male participants). The findings were similar and statistically significant correlations ranging from 0·20 (95% CI 0·07–0·32 for p-Tau-217 concentrations) to 0·29 (0·17–0·40 for Nfl concentrations) were observed only between mothers and offspring (appendix 1 p 17).

The effect of parental BBM on the BBM of the offspring was evaluated using multiple linear models, which included variables that were shown to be associated with the BBM concentrations of the G1 participants in the previous analysis (appendix 1 pp 18–19). The results supported those observed using partial correlations, and statistically significant standardised effect sizes of the BBM of the parents ranged between 0·12 and 0·25 SD units of standardised BBM, corresponding to the effect size of 3–13 years on the offspring's age (appendix 1 pp 18–19). *APOE* ε4 carrier status was not associated with the BBM or the offspring, whereas the strongest effect sizes were observed for serum creatinine concentration, especially for daughters (pTau-217, GFAP, and NfL concentrations) and alcohol usage for sons (pTau-217 concentration).

Discussion

BBMs are emerging as a potential diagnostic tool for dementing diseases, but it is important to understand confounding factors and, ultimately, to establish cutpoints for abnormal BBM concentrations.[3,7] Our population-based

	β estimate (95% CI)	p value	R^2
Amyloid β42:40			
Full model	..	..	0·148
Age, years	−0·029 (−0·032 to −0·026)	<0·0001	0·140
Sex			
Male	−0·087 (−0·169 to −0·005)	0·036	0·002
Female	Reference category		
BMI, kg/m^2	−0·018 (−0·026 to −0·010)	<0·0001	0·010
pTau-217			
Full model	..	..	0·272
Age, years	0·040 (0·037 to 0·043)	<0·0001	0·265
Sex			
Males	0·163 (0·087 to 0·238)	<0·0001	0·009
Females	Reference category		
BMI, kg/m^2	−0·019 (−0·027 to −0·012)	<0·0001	0·013
GFAP			
Full model	..	..	0·624
Age, years	0·060 (0·058 to 0·062)	<0·0001	0·612
Sex			
Males	−0·210 (−0·264 to −0·155)	<0·0001	0·027
Females	Reference category		
BMI, kg/m^2	−0·030 (−0·035 to −0·025)	<0·0001	0·058
NfL			
Full model	..	..	0·608
Age, years	0·060 (0·058 to 0·062)	<0·0001	0·601
Sex			
Males	0·010 (−0·046 to 0·065)	0·73	0·001
Females	Reference category		
BMI, kg/m^2	−0·031 (−0·036 to −0·025)	<0·0001	0·058

Values are β estimates, their 95% CIs, and p values from linear models. BBM are Z-score standardised. Therefore, the β estimate expresses the effect size in SD units for a given BBM. Age and sex are entered simultaneously into the model. R^2 values represent the goodness of fit of each full model. For each individual variable (age, sex, and BMI), R^2 values indicate the partial R^2 values. BBM=blood-based biomarkers. GFAP=glial fibrillary acidic protein. NfL=neurofilament light chain. pTau-217=phosphorylated tau 217.

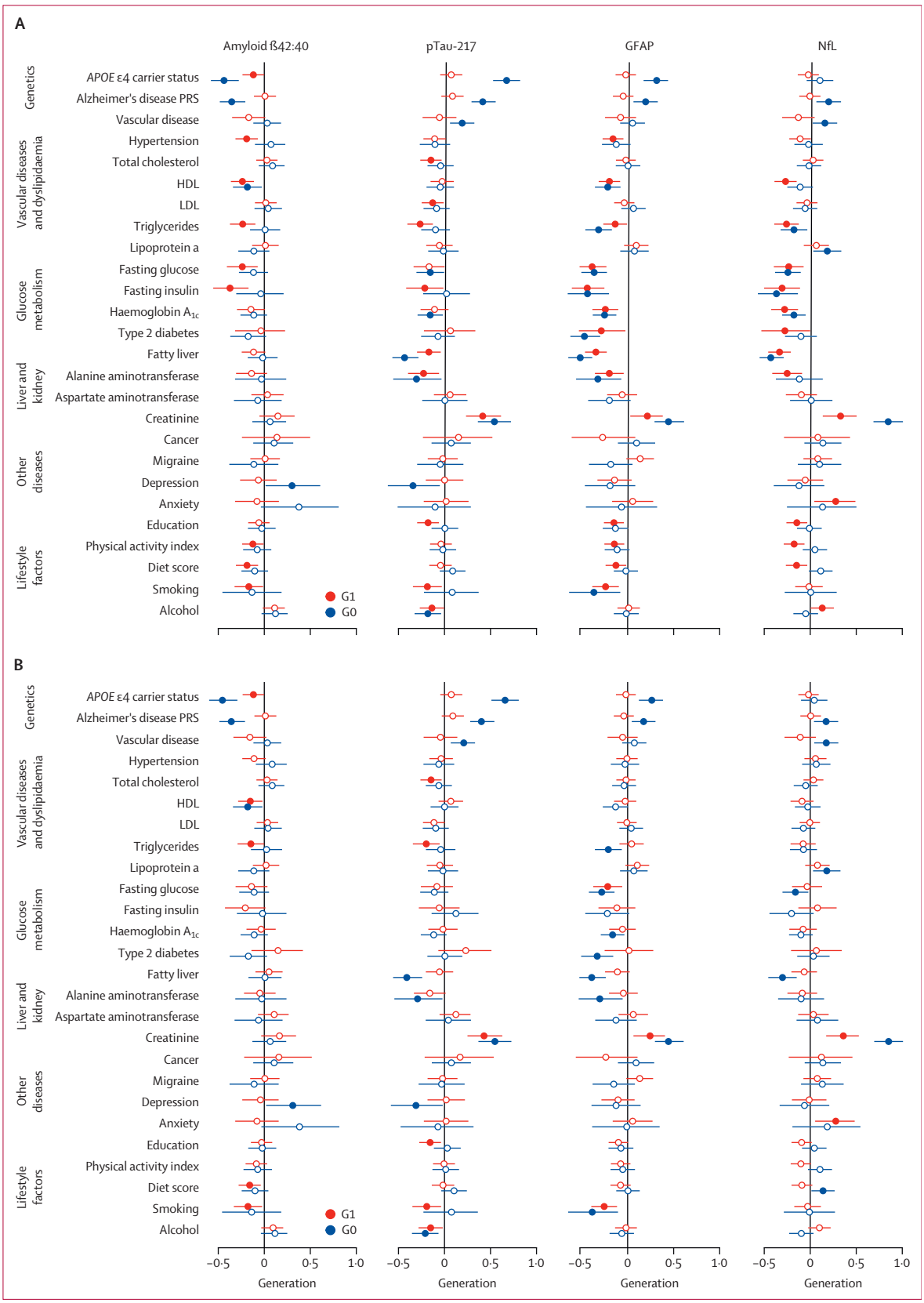
Table 2: **Association of BBM with age, sex, and BMI in the whole study population**

study focuses on adults aged 41–90 years, thereby describing BBM concentrations additionally at younger ages, when clinically detectable cognitive impairments are rare but the disease processes might have already begun. Studies using a similar ALZpath pTau-217 assay as in our study on participants with varying stages of cognitive impairment suggested cutpoints for amyloid β positivity to be 0·42 pg/mL[16] or 0·475 pg/mL.[38] In our study, 300 (15%) of the participants had pTau-217 concentrations higher than 0·42 pg/mL and 234 (11%) had concentrations higher than 0·475 pg/mL. The majority of these participants (218 [73%] and 177 [76%], respectively) were older than 70 years with probable ongoing neuropathology, but some participants in middle age exceeded these cutpoints without having abnormal values in other BBM. Although pTau-217 could indicate early pathology in these individuals in middle age, high values might also be caused by the short-term fluctuation as detected by Brum and colleagues[39] in their 10-week-long trial among cognitively unimpaired individuals aged 40–60 years. These findings suggest that individuals with a single high BBM concentration should be followed up and retested for clinical decision making. For NfL, age-specific cutpoints were defined in a study population of 5–90 years.[40] Our NfL concentrations appear to be somewhat higher than these suggested cutpoints, but similar to NfL measures reported in another, smaller study in a Scandinavian population.[41] Discrepancies could reflect differences in study populations, eg, regarding their cognitive function status or comorbidities.

We analysed a wide array of factors to identify those that are associated with BBM. Similarly to previous literature, *APOE* ε4 carrier status showed robust association with higher pathology in amyloid β42:40 ratio,[23,28] pTau-217,[42] and GFAP concentrations. Interestingly, these associations were not apparent in G1 participants but had a clear effect size in G0. Alzheimer's disease PRS followed the same trend. Although *APOE* ε4 is the best-established genetic risk factor for late-onset Alzheimer's disease,[43] our data suggest that genetic risk factors do not start to have a role in BBM accumulation until older age, although individuals homozygous for *APOE* ε4 might already have decreased amyloid β42:40 ratio in midlife.

Our observations on serum creatinine concentrations are in line with the literature reporting that chronic kidney disease or poor kidney function is associated with higher pTau-217,[25,42,44] GFAP,[23,25] and NfL[23–25,27,28] in diverse populations, whereas for amyloid β42:40 ratio, previous results were mixed.[23,28,45] Renal dysfunction appears to be a significant confounder for BBM measures, and might exert an effect on BBM concentrations due to physiological processes by reducing the clearance of proteins in the blood rather than being a risk factor for Alzheimer's disease pathology.[25] There are discrepancies in the research on whether chronic kidney disease should be taken into account[42,44] or not[15,25] when predicting development of dementia using p-tau217 concentration. Discrepancies can be related to differences in study populations. For instance, in the study by Bornhorst and colleagues,[44] 43% of participants had chronic kidney disease. In our study, 142 (17%) of G0 participants and 113 (9%) of G1 participants had creatinine values outside the target range (females ≥90 μmol/L, males ≥100 μmol/L). Further studies, with a deeper evaluation of kidney function measuring, for instance glomerular filtration rate with or without cystatin C, are needed to investigate whether renal dysfunction should be taken into account when determining the cutpoints for abnormal BBM to avoid possible misdiagnosis of Alzheimer's disease in patients with chronic kidney disease.

Many factors related to dyslipidaemia, impaired glucose metabolism, and decreased liver function were associated with BBM. After adjusting for BMI, the association of many of these factors, which are often correlated with obesity, became non-significant especially in the G1 generation, but to a lesser extent in the G0 generation. Increased BMI might be a confounder among participants in middle age whereas



sizes express how many SD units a given BBM changes when having an adverse condition compared with a healthier condition. For the amyloid β42:40 ratio, negative effect sizes indicate adverse associations, and for pTau-217, GFAP, and NfL, positive effect sizes indicate adverse associations. Filled circles represent statistically significant effect sizes and open circles non-significant effect sizes. BBM=blood-based biomarkers. GFAP=glial fibrillary acidic protein. HDL=high-density lipoprotein. LDL=low-density lipoprotein. NfL=neurofilament light chain. pTau-217=phosphorylated tau 217. PRS=polygenic risk score.

Figure 2: **Associations of different variables with BBM concentrations adjusted for age and sex (A; model 1) and adjusted for age, sex, and BMI (B; model 2)**
A separate linear model was created for each variable. BBMs are *Z* score standardised. All variables are dichotomised into value 0 (indicating the reference, no diseases, laboratory values within reference, and a healthy lifestyle) and value 1 (disease, laboratory values outside reference values, and an unhealthy lifestyle). Therefore, the effect

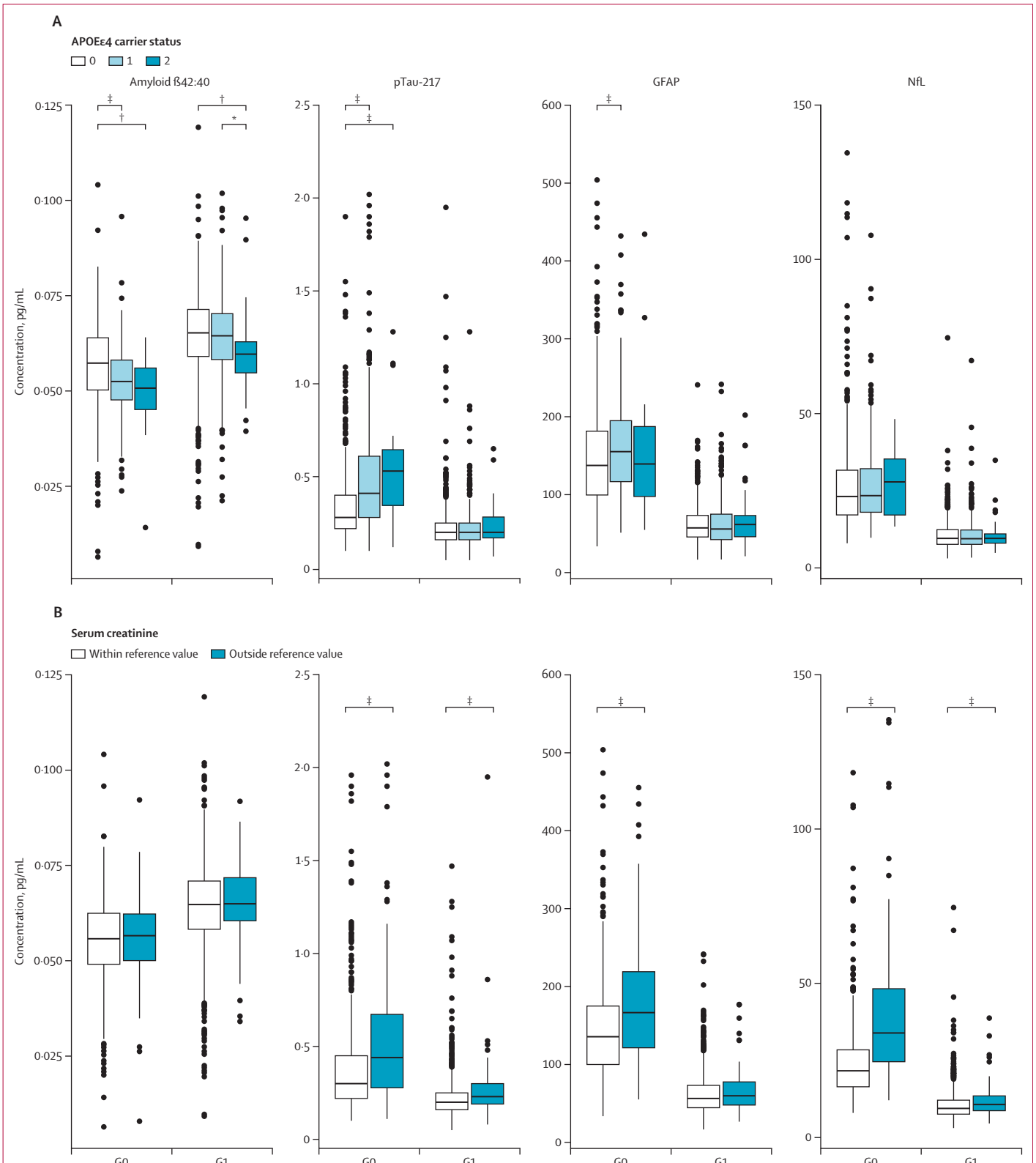


Figure 3: **Distribution of BBM within generations G0 and G1 for different APOEε4 carrier status (A; zero, one, or two alleles) and serum creatinine concentration within or outside reference value (B)** Boxplots are based on untransformed data, but the difference between the generations are tested using logarithmic transformations for skewed BBM (pTau217, GFAP, and NfL). Statistically significant differences between the groups within generations are marked with an asterisk. *p<0·05 for the difference between the groups. †p<0·01 for the difference between the groups. ‡p<0·001 for the difference between the groups. BBM=blood-based biomarkers. GFAP=glial fibrillary acidic protein. NfL=neurofilament light chain. pTau-217= phosphorylated tau 217.

	Amyloid β42:40		pTau-217		GFAP		NfL	
	r (95% CI)	p value	*r* (95% CI)	p value	*r* (95% CI)	p value	*r* (95% CI)	p value
Mother and daughter	−0·06 (−0·17 to 0·06)	0·31	0·07 (−0·05 to 0·18)	0·24	0·28 (0·16 to 0·38)	<0·0001	0·17 (0·05 to 0·28)	0·0043
Mother and son	0·02 (−0·11 to 0·15)	0·78	0·21 (0·08 to 0·34)	0·0015	0·21 (0·08 to 0·34)	0·0018	0·35 (0·23 to 0·46)	<0·0001
Father and daughter	−0·06 (−0·21 to 0·09)	0·42	0·02 (−0·12 to 0·17)	0·75	0·22 (0·08 to 0·35)	0·0032	0·07 (−0·07 to 0·22)	0·33
Father and son	−0·01 (−0·18 to 0·16)	0·92	0·03 (−0·15 to 0·20)	0·76	0·09 (−0·08 to 0·26)	0·29	0·06 (−0·11 to 0·23)	0·48

BBM=blood-based biomarkers. GFAP=glial fibrillary acidic protein. NfL=neurofilament light chain. pTau-217=phosphorylated tau 217.

Table 3: **Sex-specific partial correlations controlling for the age of the parent and offspring for each BBM**

possibly pathological processes might be involved in old age. For instance, although certain BBMs might be associated with liver function,[46] hepatic and renal dysfunction often coexist and might jointly influence BBM concentrations.[47,48] The fact that BMI negated many of the observed associations is intriguing. Previous studies have reported that high BMI might be associated with lower BBM concentrations.[24–26] This finding is thought to be because of increased blood volume caused by obesity, which could dilute BBM concentrations that are already small compared with traditional laboratory measures. On the other hand, midlife obesity is a risk factor for later dementia, but weight is often lost during the preclinical phase of dementia which could imply reverse causality in the BMI–dementia association.[49] Although our study suggests that BMI is an important factor that might modify BBM measures, it remains unclear whether BMI acts as a confounder or whether it is involved in the Alzheimer's disease pathological process. Moreover, the role of BMI might differ by age.

The associations in lifestyle factors with BBM have previously gained less attention and the existing literature is inconsistent. In agreement with our study, GFAP has been reported to be lower in former smokers than never smokers.[48] Higher GFAP concentrations are reported with increased alcohol usage[23] whereas in our study, alcohol usage was associated with lower pTau-217 concentrations. On the other hand, no association between smoking or alcohol usage and other BBMs have been reported.[23,24] Inconsistent findings might be explained by different methods to assess these factors, and self-reported questionnaires might not reflect the true behaviour. For instance, in our study, former smokers were included in the same category with never smokers and participants evaluated their current alcohol consumption, which might not reflect the usage history and, thus, might affect the results. From a cross-sectional perspective, the role of lifestyle factors on BBM concentration seems to be small and findings inconsistent, but further studies with cumulative exposures could shed more light on their effect on BBM.

Although family history is one of the strongest risk factors for late-onset Alzheimer's disease,[29–31] little is known about the intergenerational associations of BBM. Our intergenerational associations are in line with a study done in male twins aged 60–73 yeas in which amyloid β40, amyloid β42, total tau, and NfL concentrations were heritable, but amyloid β42:40 ratio was not and was largely explained by non-shared environmental effects and measurement error.[36] *APOE* ε4 carrier status and Alzheimer's disease PRS were not associated with BBM among participants in middle age, suggesting that intergenerational associations could be explained by other factors besides genetics. In accordance with our results, previous studies have suggested that maternal family history of Alzheimer's disease might increase offspring's risk more than paternal history of disease.[32–35] This finding might be caused by sociocultural reasons predisposing women to lower education in older generations, longer life expectancy of females leading to increased prevalence of Alzheimer's disease, and biological reasons, such as the maternal transmission of an X chromosome carrying a disproportionate density of neural genes that could affect offspring's Alzheimer's disease risk.[50] In our G0 participants, the distributions of age and years of education were similar between females and males but the number of mother–offspring pairs was higher than that of fathers. However, only maternal associations existed when repeating the analysis by including those participants who had BBM measures from both parents, further suggesting that there might be a biological reason for stronger maternal associations instead of mere increase in statistical power.

Our study has several strengths and weaknesses. We included two distinct age groups, comprising individuals in middle age, a potential target group for early prevention, and their parents, representing the age group when cognitive impairment is common. Exploring intergenerational associations of BBM is a novel aspect to the current research. The cohort is well described with detailed data on comorbidities and lifestyle factors, enabling an extensive investigation of the role of various confounders on BBM. However, information about some of the comorbidities and lifestyle factors were based on self-administered questionnaires and could be subjected to reporting bias. Moreover, some of the investigated factors presented heterogeneity in disease causes: vascular diseases comprised a variety of conditions that might interfere with the cerebral blood circulations, and cancer comprised different types of cancers. Stroke and cerebral haemorrhage were included into the vascular diseases because of the small number of cases among our study population. Our present study is cross-sectional, and longitudinal settings are needed to shed more light on the cumulative effect of confounders on the level and change in

BBM. We did not invite individuals with previously diagnosed dementia and, unfortunately, we did not do a clinical assessment of cognitive function among the study participants. It is therefore possible that especially some of the older participants could have had some degree of cognitive impairment.

BBMs might soon revolutionise the clinical diagnosis of Alzheimer's disease and related dementias. We identified several comorbidities and confounders that might influence BBM concentrations, parental transmission being one of them. Our study could be applied to generate hypotheses for further studies investigating the magnitude of effects the identified confounders have in clinical practice, and to determine which factors directly contribute to the amyloid β pathology and which factors act through other physiological processes and might lead to misdiagnosis of Alzheimer's disease. Our study contributes to the much-needed research on diverse, well characterised populations to establish cutpoints for BBM and to decide whether they should be modified for certain conditions, such as kidney disease or obesity.

Contributors

All authors contributed to the study conception and design. Data collection was done by KP, MJ, MK, EJ, TPL, PT, JV, OR, and SPR. Material preparation was done by AL-P, H-MP, KB, and HZ, and the statistical analysis by MAH. The first draft of the manuscript was written by MAH, and JM and SPR assisted in drafting the manuscript. All authors commented on previous versions of the manuscript and read and approved the final manuscript. MAH and SPR had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. MAH, AL-P, H-MP, KB, HZ, and SPR accessed and verified the underlying blood-based-biomarker data reported in this study.

Declaration of interest

MJ has received lecture fees from Amgen, AstraZeneca, Boehringer Ingelheim, NovoNordisk, and Novartis. EJ is a board member of the Finnish Foundation for Cardiovascular Research. H-MP has received payment and travel support from Roche Diagnostics for a short presentation in EuroMedLab 2023. HZ has served at scientific advisory boards and/or as a consultant for AbbVie, Acumen, Alector, Alzinova, ALZpath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Quanterix, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, and has given lectures sponsored by Alzecure, BioArctic, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, Roche, and WebMD. HZ is Chair of the Alzheimer's Association Global Biomarker Standardization Consortium and Chair of the IFCC WG-BND. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abbvie, AC Immune, ALZpath, Aribio, Beckman Coulter, BioArctic, Biogen, Eisai, Neurimmune, Ono Pharma, Sanofi, Julius Clinical, Lilly, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers. HZ and KB are cofounders of Brain Biomarker Solutions in Gothenburg, which is a part of the GU Ventures Incubator Program. All other authors declare no competing interests.

Data sharing

Data from the Cardiovascular Risk in Young Finns Study (YFS) can be provided by the YFS Steering Group and Data Sharing Committee pending scientific review and a completed material transfer agreement. Requests for the YFS data should be submitted to the Principal Investigator of YFS, OR (olli.raitakari@utu.fi).

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