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ORIGINAL RESEARCH

Platelet Function Is Associated With Dementia Risk in the Framingham Heart Study

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BACKGROUND: Vascular function is compromised in Alzheimer disease (AD) years before amyloid and tau pathology are detected and a substantial body of work shows abnormal platelet activation states in patients with AD. The aim of our study was to investigate whether platelet function in middle age is independently associated with future risk of AD.

METHODS AND RESULTS: We examined associations of baseline platelet function with incident dementia risk in the community-based FHS (Framingham Heart Study) longitudinal cohorts. The association between platelet function and risk of dementia was evaluated using the cumulative incidence function and inverse probability weighted Cox proportional cause-specific hazards regression models, with adjustment for demographic and clinical covariates. Platelet aggregation response was measured by light transmission aggregometry. The final study sample included 1847 FHS participants (average age, 53.0 years; 57.5% women). During follow-up (median, 20.5 years), we observed 154 cases of incident dementia, of which 121 were AD cases. Results from weighted models indicated that platelet aggregation response to adenosine diphosphate 1.0 µmol/L was independently and positively associated with dementia risk, and it was preceded in importance only by age and hypertension. Sensitivity analyses showed associations with the same directionality for participants defined as adenosine diphosphate hyper-responders, as well as the platelet response to 0.1 µmol/L epinephrine.

CONCLUSIONS: Our study shows individuals free of antiplatelet therapy with a higher platelet response are at higher risk of dementia in late life during a 20-year follow-up, reinforcing the role of platelet function in AD risk. This suggests that platelet phenotypes may be associated with the rate of dementia and potentially have prognostic value.

Key Words: aggregation ■ Alzheimer's disease ■ dementia ■ Framingham ■ LTA ■ platelet function ■ risk prediction

Alzheimer disease (AD) is the most important form of dementia and its growing prevalence requires biomarkers that can identify AD risk as early as middle age, when preventive interventions will be more effective.[1,2] Recent studies suggest that vascular function is compromised in AD years before amyloid-beta (Aβ) and tau abnormalities can be detected,[3–5] and platelet activation is one of the earliest events observed in capillary dysfunction.[6] Prior work also suggests that platelets play a functional role in amyloid plaque formation in experimental animal models,[7–9] and both abnormal platelet activation and fibrinogen-amyloid

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CLINICAL PERSPECTIVE

What Is New?

- Using one of the largest platelet function repositories available at the Framingham Heart Study, we explored associations of the platelet aggregation response measured by light transmission aggregometry and the risk of developing subsequent dementia during a 20-year follow-up.
- Our study shows middle-aged individuals free of antiplatelet therapy with a higher platelet aggregation response to 1.0 μmol/L adenosine diphosphate are at higher risk of developing dementia.
- Associations with the same directionality were observed for the response to both adenosine diphosphate and epinephrine, suggesting that the associations are not agonist-specific.

What Are the Clinical Implications?

- Prior research has shown that platelets participate in beta-amyloid and tau physiology, potentially contributing to Alzheimer disease pathology, however, whether abnormalities in platelet function are associated with the risk of developing Alzheimer disease later in life is unknown.
- Our study reinforces a role of platelet function in Alzheimer disease, suggesting that platelet phenotypes may be associated with the rates of developing dementia and potentially have prognostic value.
- Future methodological innovations for large-scale exploration of platelet function in at-risk populations are needed.

Non-standard Abbreviations and Acronyms

FHS	Framingham Heart Study
LTA	light transmission aggregometry

hemostasis interactions have been reported in patients with AD.[10–14]

The central role of platelets in cardiovascular disease (CVD) is well established, and platelet function is the target of drug treatments to prevent arterial thrombosis. Longitudinal studies have consistently demonstrated an association between platelet function and CVD events in patients with established coronary artery disease,[15–17] and the role of platelet function in predicting incident CVD events and mortality, remaining independently significant after adjusting for traditional risk factors, has been described in a healthy population from the FHS (Framingham Heart Study).[18] Recently, investigators using the FHS demonstrated an independent role of cardiovascular risk profiles on the risk of incident dementia when combined with genetic risk profiles.[19] Whether an abnormal platelet activation state is associated with a heightened risk of AD later in life is still unknown.

The FHS is a longitudinal, prospective, community-based cohort with long-term surveillance that contains a large number of individuals who are free of antiplatelet therapy and with laboratory-based measures of platelet function. The aim of this study was to examine whether baseline platelet function was independently associated with incident dementia in the FHS.

METHODS

All participants provided written informed consent. Study protocols and consent forms were approved by the institutional review board at the Boston University Medical Center. All data and materials have been made publicly available at the BioLINCC repository and can be accessed at https://biolincc.nhlbi.nih.gov/home/. Code used for analysis is available upon reasonable request and for collaboration and reproducibility purposes.

Framingham Heart Study

The FHS is one of the oldest active longitudinal cohort studies in the United States, initiated in 1948 and with over 70 years of follow-up in the baseline cohort. The original cohort had 5209 residents of Framingham, MA, who after recruitment underwent up to 32 examinations, every 2 years, where a variety of clinical and laboratory data were collected.[20] In 1971, a total of 5124 offspring of the original cohort and their spouses were enrolled in the 'offspring' cohort. A total of 9 examinations are available in the offspring cohort, with the latest completed examination performed in 2011 to 2014.[21] Offspring cohort participants attending the fifth examination cycle (1991–1995), during which platelet function was assayed, were eligible for the present investigation.

Platelet Aggregation Data

Platelet aggregation was previously characterized, and the methods have been described.[18] A total of 3799 individuals attending the fifth examination cycle of the offspring cohort were considered. Excluded observations were those when the use of aspirin was reported at the time of platelet function analyses, as determined by lack of platelet response to arachidonic acid. Briefly, platelet aggregation was evaluated in fresh citrated blood samples in isolated platelet rich plasma in

response to adenosine diphosphate (ADP) and epinephrine by light transmission aggregometry (LTA) with a 4-channel PAP-4 aggregometer (Bio/Data, Horsham, PA). Our analysis primarily focused on the platelet aggregation response to ADP 1.0 µmol/L because of its association with CVD outcomes in our previous study[18] and larger number of observations available. As a secondary analysis, the aggregation response to ADP 3.0 and 5.0 µmol/L, and epinephrine at 0.1, 0.5, 1.0, and 3.0 µmol/L were also explored. Participants who responded (≥50% maximal aggregation) at least at 1 low dose of ADP (0.05, 0.1, 0.5, and/or 1.0 µmol/L) were considered hyper-responders for ADP. Similarly, hyperreactivity to epinephrine was defined as ≥50% maximal aggregation with at least 1 low dose of epinephrine (0.01, 0.03, 0.05, 0.1, 0.5, or 1.0 µmol/L).

Dementia Surveillance

Dementia characterization methods in the FHS were made in accordance with Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria and FHS methods for continuous surveillance of dementia have been previously described.[22–25] Cognition was examined every cycle with the use of the Mini-Mental State Examination scale.[26] The Mini-Mental State Examination was used to identify participants for dementia screening when performance fell below education-based cutoff scores at any examination; there was a decline of >3 points between consecutive examinations, or >5 points from the participant's highest obtained Mini-Mental State Examination score. Participants were also flagged for further evaluation in response to referrals or concerns from participants themselves, relatives, or other professionals. Flagged participants were offered a full neuropsychological test battery and a neurological examination, which were reviewed to refer for dementia review. The dementia review panel, which includes neurologists and neuropsychologists, reviews possible cognitive decline and dementia and determines whether a participant had dementia, the dementia subtype, and the date of diagnosis using data from multiple sources. After a participant dies, a medical panel manually reviews medical records up to the date of death and includes an assessment of whether the participant might have had cognitive decline since his or her last examination. This medical panel refers any participants who might have had cognitive decline to the dementia review panel for postmortem review. The main outcome of our study was incident dementia using continuous surveillance with clinician diagnosis at the end of the follow-up period up to 2018. A sensitivity analysis for confirmed diagnosis of AD was also conducted.

Covariates

Baseline was defined as the time of clinic examination corresponding to the platelet function detection (examination cycle 5). Smoking was defined based on smoking status the year preceding baseline. We defined hypertension as a systolic blood pressure ≥130 mm Hg and/or use of antihypertensive drugs.[27] Diabetes was identified by fasting glucose levels >126 mg/dL (7.0 mmol/L) and/or use of diabetes treatments. Levels of all cardiovascular risk variables including body mass index, total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined from examination cycle 5. Years of education were included for each participant. History of cardiovascular disease (CVD) events at the time of clinical examination included: reported history of coronary heart disease, congestive heart failure, myocardial infarct, intermittent claudication, ischemic stroke, intracerebral hemorrhage, or transient ischemic attack.

Statistical Analysis

Variables used in the analyses were: (A) platelet function (% maximal aggregation) in response to ADP at 1.0 µmol/L; (B) outcome: clinical dementia diagnosis; and (C) basic demographic and clinical parameters (continuous or categorical), which included age, sex, years of education, body mass index, smoking, high-density lipoprotein cholesterol, low-density-lipoprotein cholesterol, total cholesterol, triglycerides, hypertension, diabetes, and history of CVD. Demographic and clinical differences between study groups were assessed in univariate analyses using t tests for continuous variables and χ^2 tests for nominal variables. When non-normal distributions were detected, Kruskal–Wallis tests were used. Spearman correlations were used to test the association between continuous variables. In general, statistical significance was defined by $P<0.05$, and tendencies by $P\leq0.1$.

R 3.6 and Python 3.7 were used for statistical analysis and visualization. Duration of follow-up was calculated from the date of platelet function characterization until the latest clinical diagnosis available before the end of follow-up in 2018. In observational studies like the FHS where there is no random assignment to treatment groups (or variable of interest like the platelet function response in our case), the unadjusted comparison between treatment groups may be misleading because of confounding. One method to adjust for measured confounders is inverse probability of treatment weighting (IPW).[28,29] To identify potential independent associations between platelet aggregation and incident dementia, we fit Cox proportional hazard models with IPW as described previously.[30] The IPW approach weighted each subject by the inverse of the probability of each subject's observed platelet function level using the median as a

cutoff, adjusting for non-random selection of subjects into high versus low platelet function groups. These probabilities were estimated from a logistic regression model for high versus low platelet function, with adjustment for age, sex, high school education, body mass index, hypertension, diabetes, current smoking status, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, triglycerides, and history of CVD. The Cox models were then weighted by the estimated probabilities of platelet function level, with adjustment for the same covariates. The proportional hazards assumption was validated using the Schoenfeld residual test included in the cox.zph function of the coxph package.[31,32] To help interpretability and visualization of forest plots, we computed hazard ratio corresponding to an increase in LTA of 10 units (LTA=LTA/10). Spline terms on the platelet aggregation response were used to estimate the relationship between the platelet aggregation response and risk of dementia in the weighted Cox models. Next, we estimated the cumulative incidence function for dementia for the ADP hyper-responder groups (yes/no) using propensity scores and the causalCmprsk package.[28] Because of the competing risk of death, the models are interpretable as cause-specific hazard models, ie, for the risk of dementia among those still alive. Finally, in an exploratory approach we applied the survival data implementation of Breiman random-forest models in the randomForestSRC[33] to estimate the relative importance of each of the covariates in dementia risk. The variable importance of each predictor is estimated by using variable selection methods of random forest survival models. The variable selection method uses a prediction error approach by "noising-up" each variable in turn. The variable importance of a variable Xi is the difference in prediction error when Xi is randomly permuted, compared with the prediction error under the true values. The package ggRandomForests was used for visualization.

RESULTS

Study Sample

The final study sample consisted of 1847 participants from FHS and included the combination of platelet aggregation (ADP 1.0 µmol/L) and clinical and demographic covariates (Figure 1) described previously. Baseline characteristics for all participants at the time of platelet function characterization are shown in Table 1. The average age of participants in the study at baseline was 53 years (interquartile range, 47–61); 57.5% were women (1062).

Platelet Aggregation

Platelet aggregation response to 1.0 µmol/L ADP followed a bimodal distribution and the median response was 11.0. Participants showing a higher response to ADP (above median) were older, predominantly women, with higher high-density-lipoprotein cholesterol and total cholesterol, and more likely to have a history of hypertension (P<0.05) (Table 1).

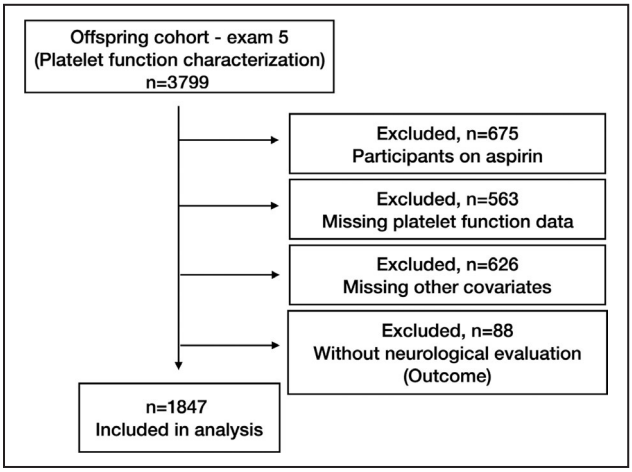

Figure 1. Inclusion diagram.

Incident Dementia

During follow-up (median, 20.5 years [interquartile range, 14.9–25.0]), 154 cases of incident dementia (102 AD cases without vascular dementia, 19 mixed dementia cases (AD+ vascular dementia), 5 vascular dementia cases (non-AD), and 28 other dementias that include frontotemporal, Lewi body, and other dementias of unknown subtype), and 269 deaths without incident dementia were observed. Logistic models for the estimation of the IPW weights indicated that age and sex were strongly associated with the platelet function response to 1.0 µmol/L ADP (Table S1). Associations with the same directionality were found when estimating weights for the other agonists and concentrations. Hazard ratios for the univariate and fully adjusted IPW multivariate Cox proportional hazard models for incident dementia are summarized in Table 2. The platelet aggregation response to 1.0 µmol/L ADP was independently associated with dementia risk (Figure 2, Table 2). Results indicated a 7% increase in dementia risk for a 10-unit increase in the response to 1.0 µmol/L ADP in the fully adjusted models. A sensitivity analysis for confirmed AD suggested results of the same directionality with the platelet aggregation response associated to higher rates of AD (Table S2). Cubic spline analysis suggested a continuous linear trajectory for the association of the response to ADP and the risk of dementia and AD both in unadjusted and adjusted models (Figure 3). Ranked feature importance of

Table 1. Baseline Demographic and Clinical Characteristics of the 1845 Men and Women in the Study Sample Grouped by Platelet Aggregation Response to 1.0 µmol/L ADP

	Overall (n=1847)	Platelet response to ADP-1 µmol/L, below median=11.0 (n=925)	Platelet response to ADP-1 µmol/L, above median=11.0 (n=922)	*P* value
Age, median [Q1, Q3], y	53.0 [47.0, 61.0]	51.0 [46.0, 60.0]	55.0 [48.0, 62.0]	<0.001
Women, n (%)	1062 (57.5)	452 (48.9)	610 (66.2)	<0.001
Years of education, mean (SD)	14.1 (2.6)	14.2 (2.6)	14.0 (2.6)	0.114
BMI, median [Q1, Q3]	26.3 [23.6, 29.3]	26.4 [23.8, 29.4]	26.2 [23.5, 29.2]	0.197
LDL cholesterol , median [Q1, Q3]	125.0 [103.5, 146.0]	124.0 [103.0, 143.0]	126.0 [104.0, 150.0]	0.11
HDL cholesterol, median [Q1, Q3]	49.0 [40.0, 60.0]	48.0 [38.0, 58.0]	50.0 [42.0, 62.0]	<0.001
Total cholesterol , median [Q1, Q3]	203.0 [179.0, 226.0]	202.0 [178.0, 221.0]	204.0 [180.0, 230.0]	0.004
Triglycerides, median [Q1, Q3]	113.0 [82.0, 163.0]	115.0 [82.0, 166.0]	112.0 [83.0, 160.0]	0.619
Smoker, n (%)	365 (19.8)	186 (20.1)	179 (19.4)	0.752
Diabetic, n (%)	48 (2.6)	20 (2.2)	28 (3.0)	0.301
Hypertense, n (%)	667 (36.1)	313 (33.8)	354 (38.4)	0.047
CVD history, n (%)	104 (5.6)	55 (5.9)	49 (5.3)	0.626
Platelet function response to ADP-1.0 µmol/L, median [Q1, Q3]	11.0 [6.0, 20.0]	6.0 [4.0, 8.0]	20.0 [15.0, 39.0]	<0.001

Definitions described in Methods. ADP indicates adenosine diphosphate; BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

random forest competing risk models assigned highest priority to age, followed by hypertension, as a distant second, immediately followed by the platelet response to 1.0 µmol/L ADP, education, and triglycerides (Figure S1). Variables with lower importance included low-density lipoprotein cholesterol, history of CVD, total cholesterol, body mass index, sex, diabetes, and smoking status. No associations were found for the higher concentrations of ADP (3.0 and 5.0 µmol/L) (Table 2). An association of ADP hyper-responders (yes/no) and higher rates of dementia was suggested for dementia and AD (Table 2, Table S2). Cumulative incidence function curves suggested that the incidence of dementia was higher in ADP hyper-responders (Figure S2), although the CIs are overlapping and there is insufficient power to conclude that the curves are different. For example, the probability of dementia before death occurring within 20 years for those non-ADP hyper-responders at baseline was 8.57% (95% CI, 6.34–8.75), while for those who are ADP hyper-responders it was 13.96% (95% CI, 8.60–16.06). An association was also found for the platelet aggregation response to 0.1 µmol/L epinephrine and dementia risk although the number of dementia cases was smaller (Table 2). An association with incident AD was also suggested for low-dose stimulation with 0.1 and 0.5 µmol/L epinephrine (Table S2). No associations were found for epinephrine hyper-responders or any of the higher concentrations of epinephrine (Table 2, Table S2). A sensitivity analysis for subgroups with AD and non-AD dementia (with and without vascular dementia) suggested a general association of platelet aggregation with any type of dementia, and not an association with AD specifically (Figures S3 and S4).

DISCUSSION

In addition to their primary role in thrombosis and hemostasis, platelets are immune cells with important inflammatory roles in both health and disease. The measurement of platelet function has gained interest as a biomarker of AD because of its potential mechanistic role. Our study in the community-based longitudinal FHS offspring cohort demonstrates that individuals with a higher response to ADP appear to be at a higher risk of dementia during a 20-year follow-up period. These findings are significant even after adjustment for a number of covariates that could play a confounding role in the association of platelet function with dementia risk.

The role of platelet function as a biomarker in AD is of interest and it has been extensively studied (reviewed in Plagg et al[34]). Platelets are cells that initiate and accelerate vascular inflammatory processes that are crucial in cerebrovascular health, but are also associated with tau and Aβ physiology, both hallmarks of AD pathology. Platelets are carriers of tau and Aβ species[35–38] and previous studies have proposed platelet-derived tau as a biomarker for AD.[39–41] Platelets are 300 to 500 times more concentrated in blood clots, compared with non-clotted blood; hence, allowing for a massive release of Aβ (either directly or as a byproduct of released amyloid-beta precursor protein [APP]) at the site of clot formation.[42] Research in experimental animal models of AD shows that platelets play a crucial

Table 2. Full List of Univariate and Fully Adjusted Hazards Ratios for the Association of Platelet Function with Incident Clinical Diagnosis of Dementia

Outcome: clinical dementia diagnosis			Univariate model		Fully adjusted model	
Platelet function measure	No. at risk	No. cases	HR (95% CI)	*P* value	HR (95% CI)	*P* value
ADP, µmol/L						
1.0	1847	154	1.10 (1.04–1.17)*	<0.001*	1.07 (1.01–1.15)*	0.03*
3.0	1847	154	1.10 (1.02–1.18)*	0.02*	1.04 (0.95–1.14)	0.36
5.0	1304	96	1.04 (0.91–1.19)	0.57	1.03 (0.87–1.21)	0.74
Epinephrine, µmol/L						
0.1	1038	98	1.10 (1.03–1.18)*	0.004*	1.09 (1.01–1.17)*	0.02*
0.5	1590	127	1.06 (1.00–1.12)*	0.07*	1.04 (0.98–1.11)	0.20
1.0	1667	124	1.02 (0.96–1.08)	0.56	1.00 (0.94–1.07)	0.93
3.0	936	61	1.01 (0.92–1.11)	0.83	1.01 (0.91–1.12)	0.80
Hyper-responders to ADP (yes/no)	1847	154	1.67 (1.06–2.61)*	0.03*	1.54 (0.94–2.52)*	0.08*
Hyper-responders to epinephrine (yes/no)	1847	154	1.25 (0.75–2.06)	0.39	1.35 (0.81–2.25)	0.25

Univariate and multivariate adjusted cytochrome C oxidase models with inverse probability weighting. Median follow-up was 20.5 years. Fully adjusted models included age, sex, high school education, body mass index, hypertension, diabetes, LDH, low-density lipoprotein, total cholesterol, triglycerides, current smoking status, and history of cardiovascular disease. Analysis excluded participants on aspirin at the time of platelet function determination. ADP indicates adenosine diphosphate; and HR, hazard ratio.

*Results with $P \leq 0.1$ are highlighted.

role in Aβ brain accumulation and vascular damage at early stages.[8,9,43] Furthermore, studies have shown platelets are responsible for the accumulation of Aβ in blood clots inside and around cerebral blood vessels in mouse models.[42] In addition, platelets induce the conversion of soluble Aβ to toxic aggregated species.[43,44] There is also commonality between the proteomic signature of the human brain with cerebral atherosclerosis, which can produce platelet activation, and AD pathology.[45] It is therefore feasible that

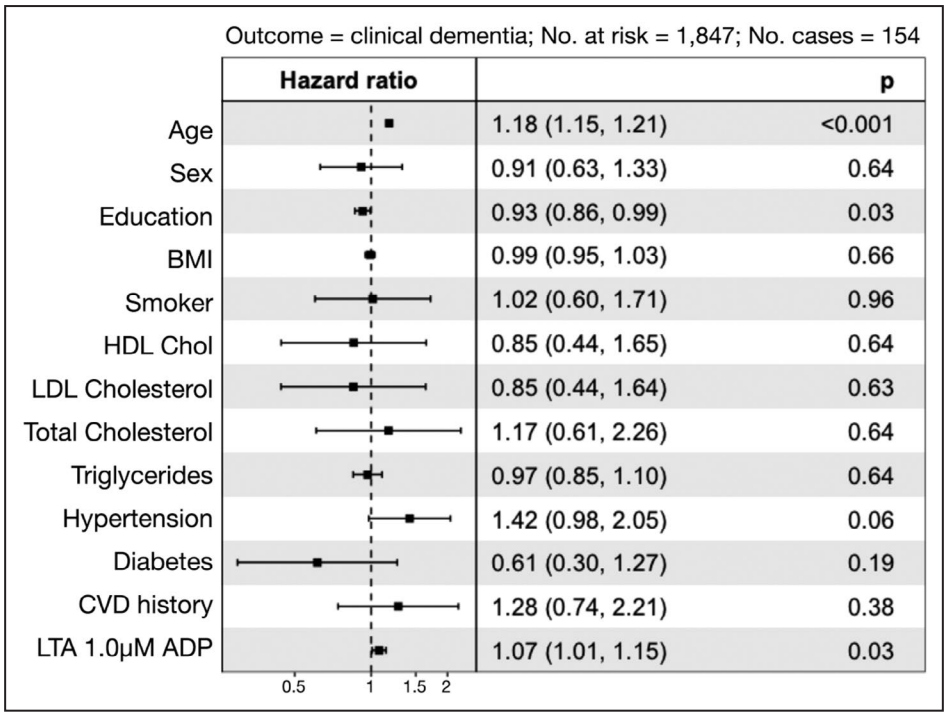

Figure 2. Forest plot for the association of platelet aggregation response to 1.0 µmol/L adenosine diphosphate and dementia risk in the FHS (Framingham Heart Study) using inverse probability of treatment weighting Cox proportional hazards regression.
ADP indicates adenosine diphosphate; BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and LTA, light transmission aggregometry.

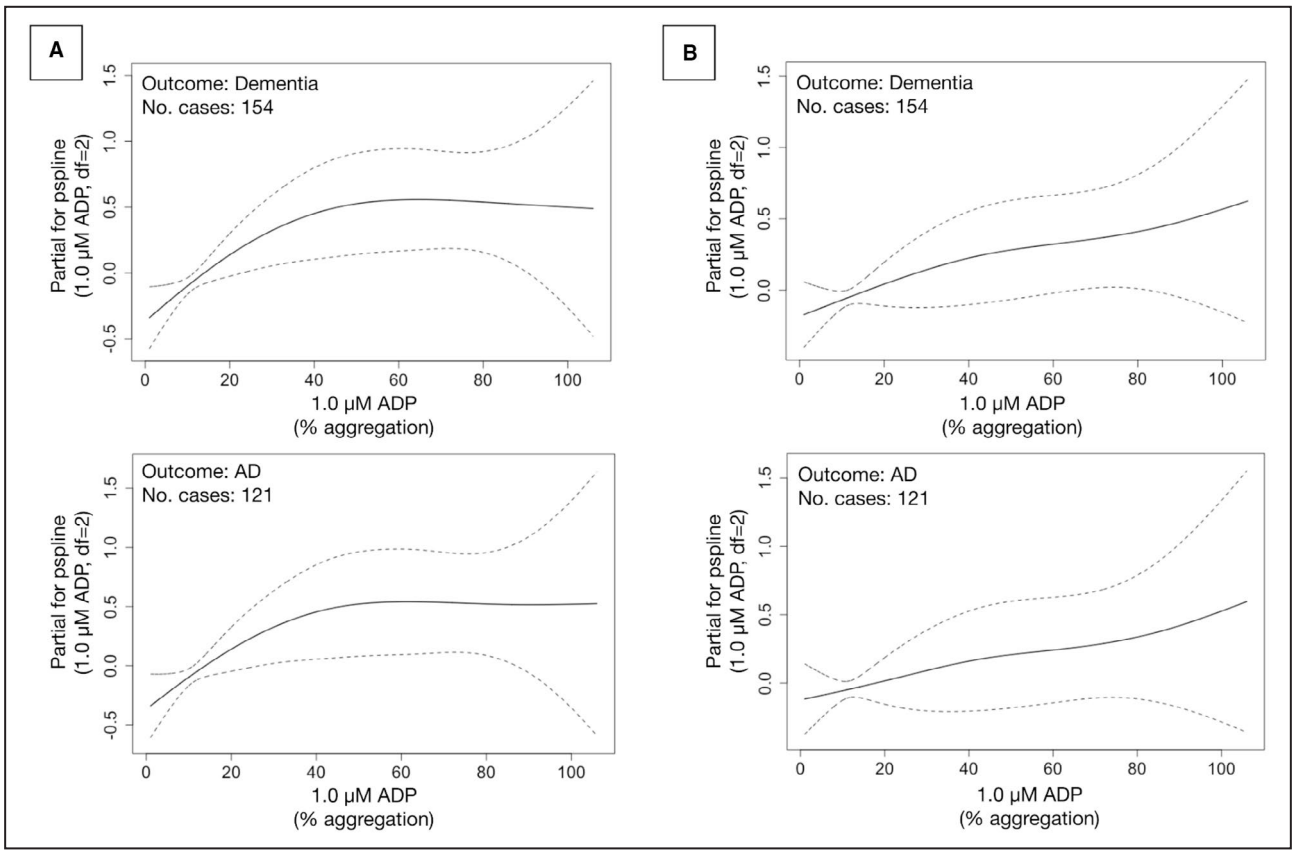

Figure 3. Unadjusted (A) and fully adjusted (B) splines of the platelet aggregation response to 1.0 µmol/L adenosine diphosphate against hazard ratio (with 95% confidence limits) for dementia and Alzheimer disease.
AD indicates Alzheimer disease; ADP, adenosine diphosphate; df, degrees of freedom; and pspline, penalised smoothing spline.

platelets could be relevant contributors to AD pathology and that an early abnormal platelet activation state precedes AD-vascular dysfunction.

Our study in 1847 participants of a well characterized community-based cohort shows an independent association of platelet function with future dementia, suggesting that platelet phenotypes may be associated with the rates of dementia and have prognostic value. Platelet aggregation can be initiated via various pathways and therefore several agonists can be used to detect platelet aggregation in functional assays, one of the most common being ADP.[46] Our results identified higher dementia risk with greater responses to ADP that was further validated with the response to epinephrine indicating that abnormal platelet phenotypes in a community-based sample who are free of antiplatelet therapy are associated with dementia risk and this deserves further study. Of special note is that the associations were significant for low-dose stimulation (1.0 µmol/L ADP and 0.1 µmol/L epinephrine) which have been previously shown to efficiently identify hyper-reactive phenotypes and outcomes.[18,47] The results of sensitivity analysis for subgroups with AD and non-AD dementia suggested a general association of platelet aggregation with any type of dementia, and not an association with AD specifically that should be evaluated in the future when more cases become available.

Discrepant results have prevented establishing a robust platelet-derived AD biomarker, probably because of technical challenges associated to platelet function detection, as well as differences in study design and measurements.[48–50] Data suggestthat peripheral platelets could be abnormally activated in early and/or preclinical stages of AD. Maccioni et al. has repeatedly proposed platelet-tau as a biomarker for AD indicating that platelet tau/p-tau ratio correlates with cognitive impairment,[39] and that the ratio of high molecular weight tau/low molecular weight tau in platelets correlates with regional brain atrophy.[51] Previous research by Ahn et al, using flow cytometry identified a heightened platelet activation state in AD compared with controls.[10] These results were later validated by the group of Laske et al, who additionally observed that activated glycoprotein IIb-IIIa complex and P-selectin were higher in patients with AD with fast cognitive decline compared with patients with AD with slow cognitive decline during a 1-year follow-up period.[52] Interestingly, it has also

been shown that Aβ binds the activated glycoprotein IIb-IIIa complex through its RHDS sequence, which causes integrin outside-in signaling and downstream activation of Syk and PLCr2, that ultimately promotes the release of the chaperone clusterin and ADP from platelets.[9] A recent study in an AD transgenic mouse model has also shown that the major contribution of atherosclerosis, to the risk of developing AD pathology, is via its effects on blood coagulation and the formation of platelet-mediated Aβ aggregates which compromise cerebral blood flow and therefore neuronal function.[43]

Meanwhile, other studies explored potential proxies of platelet function such as mean platelet volume, or markers of platelet enzymatic activity such as APP expression, the APP ratios, BACE1, ADAM-10, or cytochrome C oxidase, among others.[34] Still, to our knowledge, no studies have explored whether platelet function is associated with future risk of dementia. Several limitations may have prevented this, including technical challenges associated with platelet function detection methods since most assays cannot be done using frozen samples. Despite these limitations, a recent study explored the integration of LTA data in machine-learning models for the classification of AD versus healthy controls and reported a sensitivity of 96.6% and specificity of 80% for models that included a combination of LTA, clinical markers, and micro-RNA data. Although the results suggested that platelet function data may contribute to AD biomarker panels,[14] the authors found a higher platelet response to 0.5 μmol/L ADP in healthy controls compared with AD cases, which contradicts our hypothesis that a heightened platelet function in middle age is associated with a higher risk of incident AD. Three important differences may help explain these differences. First, the cross-sectional design and use of a case control approach with a small number of observations may limit the generalizability of the findings to larger populations. Second, potential sex differences may have influenced the results, since the authors had a higher number of women in their control population (55%) compared with the AD group (45%). Because female sex has such a large effect on all platelet assays (also evidenced in our FHS study) this may explain why they identified higher platelet function in controls. Finally, about 20% to 22% adults use aspirin regularly and it is unclear if participants on aspirin were excluded, which may have significantly affected the results. Nonetheless, the same study identified higher PAC-1 (activated GP IIb/IIIa) binding to ADP stimulation in AD cases compared with controls, in agreement with our findings, suggesting that future studies will need to delineate the role of platelet function both in preclinical and clinical stages of AD, in combination with AD biomarkers and carefully account for potential sex-effects in the above associations.

Our study has some limitations. First, we observed associations for 1.0 μmol/L ADP and for 0.1 μmol/L epinephrine, however, we were unable to explore associations with even lower stimulation doses that may facilitate the identification of abnormal platelet phenotypes. Platelet aggregation assays are known to vary across laboratories because of a lack of standardization of the concentration of agonists used, thus making comparison of studies challenging. Despite the large community-based sample and long follow-up times, the modest number of incident dementia cases did not allow for the exploration of sex-specific or ethnic-specific subgroup analyses that should be considered as additional data become available.

In conclusion, platelet function in middle age in participants of the FHS who are free of antiplatelet therapy was independently associated with future incidence of clinical dementia during a 20-year follow-up. Given these associations remained significant after adjusting for a relatively high number of covariates, our study suggests that platelet phenotypes may be associated with the rates of incident dementia and thus potentially have prognostic value. Future methodological innovations for large-scale exploration of platelet function in at risk populations are needed.

ARTICLE INFORMATION

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Supplemental Material

Table S1–S2
Figure S1–S4

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SUPPLEMENTAL MATERIAL

Table S1. Results of logistic model for propensity score weight estimation for 1.0uM ADP. High versus low platelet function groups were defined using the median as a cutoff.

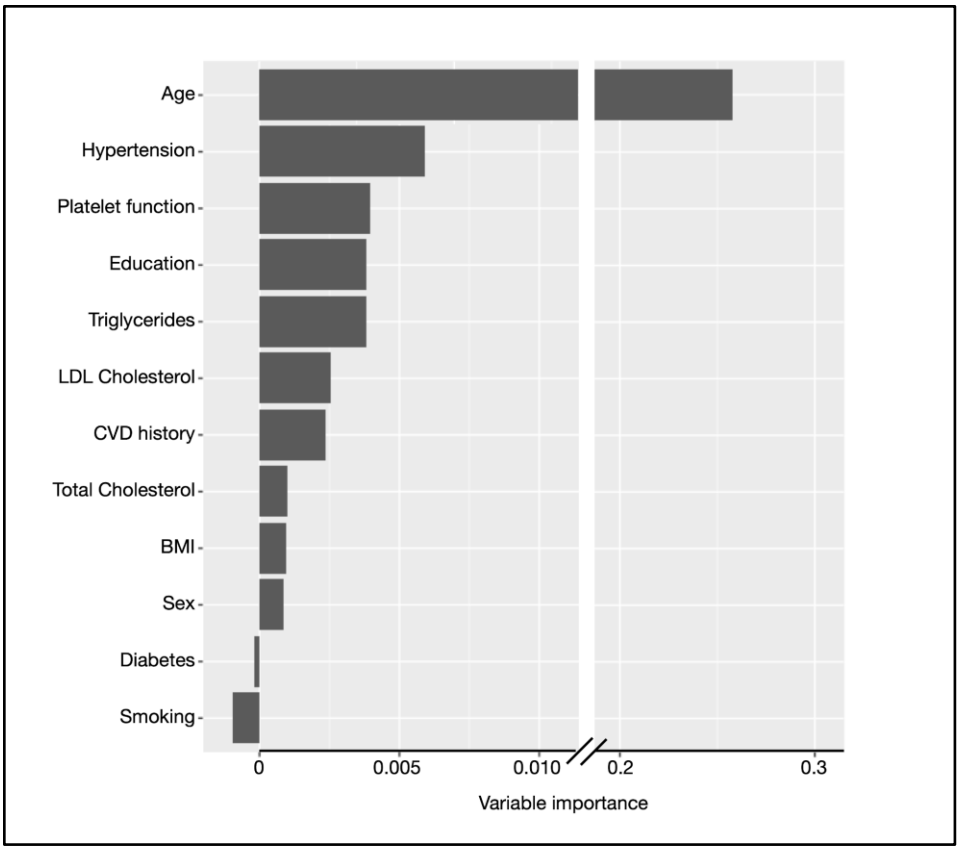
	Estimate	Std. Error	P-Value
Intercept	-2.292	0.620	0.0002
Age	**0.025**	**0.006**	**1.05e-05** ***
Female Sex	**0.686**	**0.109**	**3.14e-10** ***
Years of education	0.008	0.019	0.683
BMI	-0.001	0.011	0.965
Smoker	0.050	0.123	0.684
LDL Cholesterol	-0.001	0.004	0.862
Total Cholesterol	0.003	0.004	0.458
Triglycerides	-0.001	0.001	0.415
Hypertension	0.078	0.110	0.479
Diabetes	0.283	0.312	0.365
History of CVD	-0.202	0.212	0.340

Table S2. Full list of univariate and fully adjusted hazards ratios for the association of platelet function with incident clinical diagnosis of Alzheimer's disease (AD).

Outcome: Clinical AD diagnosis			Univariate model		Fully adjusted model	
Platelet function measure	No. at risk	No. cases	HR (95% CI)	P-Value	HR (95% CI)	P-Value
ADP, uM						
1.0	1847	121	**1.10 (1.03, 1.17)**	**<0.01**	1.06 (0.99, 1.14)	0.11
3.0	1847	121	**1.11 (1.02, 1.21)**	**0.02**	1.06 (0.95, 1.17)	0.28
5.0	1304	79	1.10 (0.94, 1.29)	0.23	1.09 (0.91, 1.30)	0.37
EPI, uM						
0.1	1038	83	**1.09 (1.01, 1.17)**	**0.03**	**1.07 (0.99, 1.16)**	**0.10**
0.5	1590	103	**1.09 (1.02, 1.17)**	**<0.01**	**1.08 (1.00, 1.15)**	**0.04**
1.0	1667	98	**1.06 (0.99, 1.14)**	**0.09**	1.05 (0.97, 1.15)	0.22
3.0	936	43	1.03 (0.91, 1.16)	0.65	1.03 (0.89, 1.18)	0.72
Hyper-responders to ADP (yes/no)	1847	121	**1.63 (0.98, 2.73)**	**0.06**	1.38 (0.79, 2.42)	0.26
Hyper-responders to EPI (yes/no)	1847	121	1.11 (0.61, 2.01)	0.74	1.19 (0.66, 2.16)	0.56

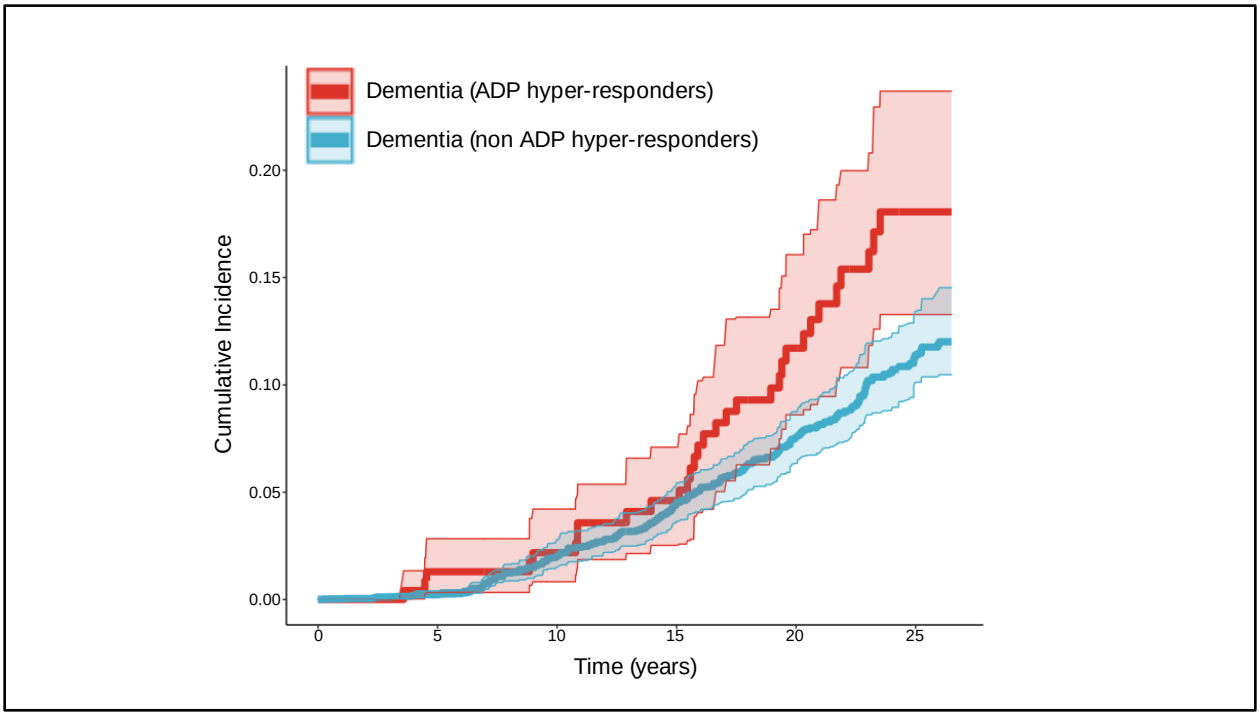
Univariate and multivariate adjusted COX models with inverse probability weighting (IPW). Median follow up was 20.5 years. Fully adjusted models included age, sex, high school education, BMI, hypertension, diabetes, LDH, LDL, total cholesterol, triglycerides, current smoking status, and history of CVD. Analysis excluded participants on aspirin at the time of platelet function determination. ADP: Adenosine diphosphate; EPI: epinephrine. Results with $P \leq 0.1$ are highlighted in bold.

Figure S1. Platelet function has predictive value in random forest survival models.



Ranked feature importance for random forest survival models for the prediction of incident dementia assigned a higher priority for platelet function response to 1.0uM ADP (coded as continuous) over other clinical covariates. Highest priority was given to age and hypertension, followed by platelet function and the remaining clinical and demographic covariates. Variable importance close to zero indicates the variable contributes nothing to predictive accuracy, and negative values indicate the predictive accuracy improves when the variable is misspecified and therefore are not informative.

Figure S2. Hyperreactivity to ADP is associated with higher rates of incident dementia in the FHS.



Adjusted cumulative incidence functions and 95% confidence intervals for dementia for ADP hyper-responders and non-ADP hyper-responders. Models were adjusted for age, sex, education, BMI, hypertension, diabetes, current smoking status, LDH, LDL, total cholesterol, triglycerides, and history of CVD (n=1,847; Events=154; Median follow up = 20.5 years).

Figure S3. Forest plot for the association of platelet aggregation response to 1.0uM ADP and dementia risk for the main dementia subgroups.

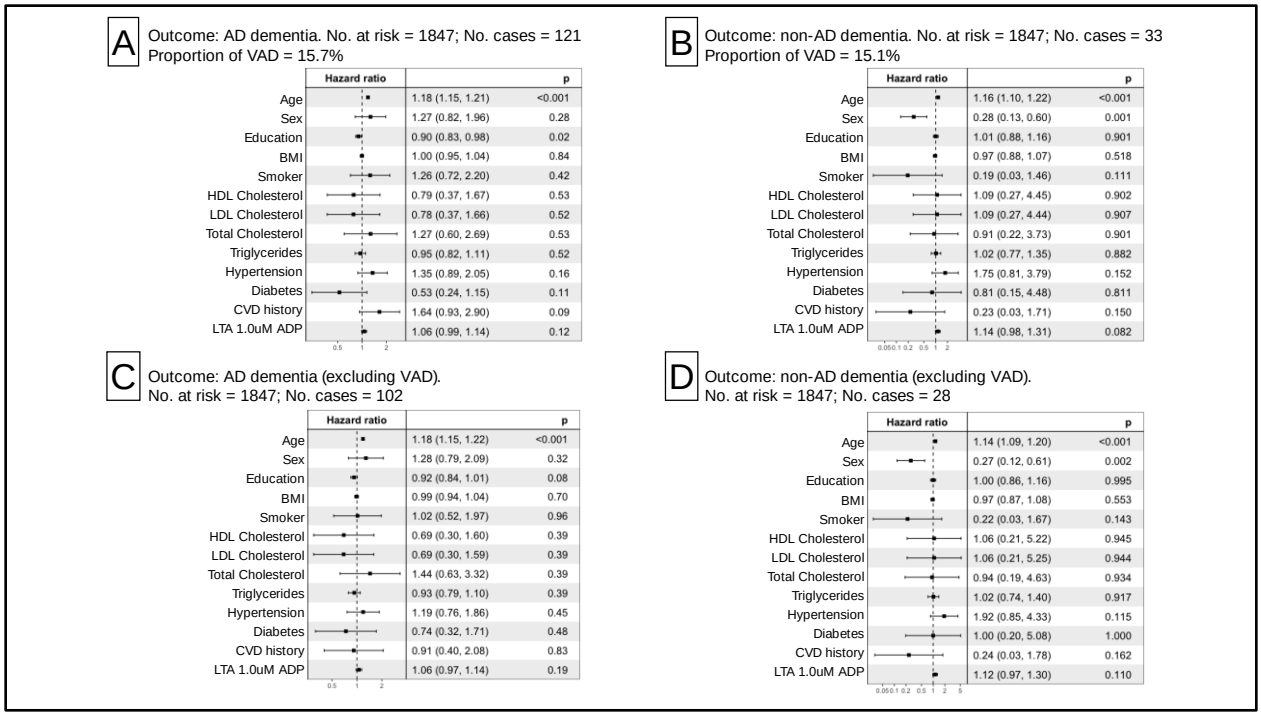
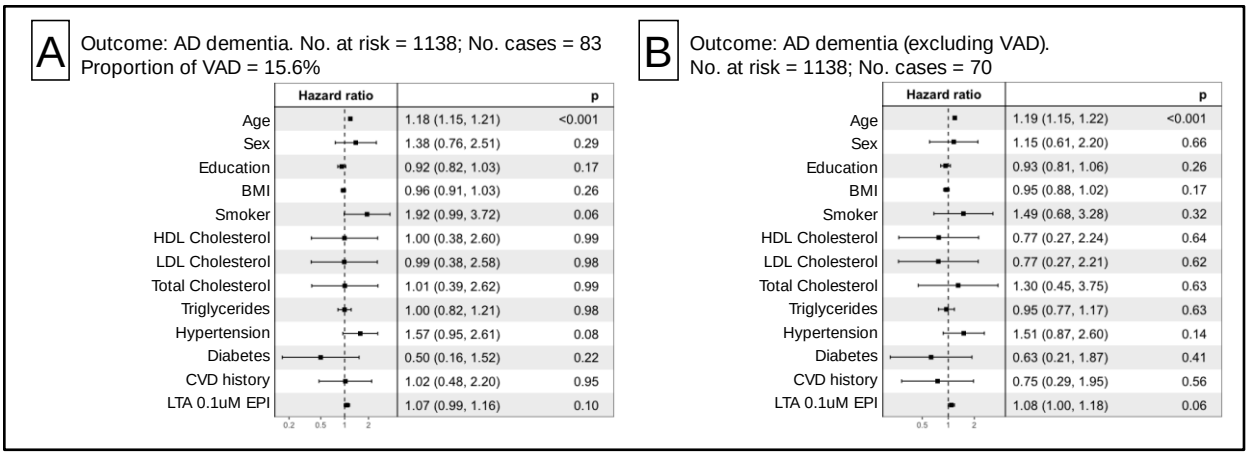


Figure S4. Forest plot for the association of platelet aggregation response to 0.1uM EPI and dementia risk for the main dementia subgroups.



An analysis against non-AD dementia (with or without VAD) was not possible due to low number of events.