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Cardiovascular correlates of platelet count and volume in the Framingham Heart Study

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Abstract

Purpose—Platelet count and volume are inexpensive, routinely-assayed biomarkers associated with cardiovascular health, but specific relationships among platelet indices, cardiovascular risk factors, and disease warrant further investigation. The purpose of this study was to understand associations among platelet count, volume, and 20 cardiovascular health-related variables in the Framingham Heart Study.

Methods—Cross-sectional analyses were performed on platelet count and volume associations with cardiovascular health indicators in three Framingham Heart Study cohorts (Original n=964, Offspring n=2,699, and Third Generation n=2,419) using multivariable linear regression analysis. Time-to-event analysis was employed for cardiovascular disease-related event incidences using Kaplan-Meier plots and Cox proportional hazards regression adjusted for age and gender.

Results—Results were concordant with the hypothesis that higher platelet counts are associated with less favorable cardiovascular risk profiles, although mean platelet volume associations were weaker. In our analysis, increased platelet count across FHS cohorts was consistently associated with smoking, triglycerides, LDL and total cholesterol levels. Some associations with platelet count appeared gender-dependent.

Conclusions—Significant associations of common blood platelet measurements are observed with gender and cardiovascular risk factors, namely smoking and lipids. Research is warranted to

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Disclosures

None.

confirm these relationships in other cohorts, evaluate differences by ethnicity and examine longitudinal effects on disease risk.

Keywords

platelets; cholesterol; cardiovascular diseases; smoking; platelet count; platelet volume

INTRODUCTION

As indicators of platelet activation and thrombosis, blood platelet count (PLT) and volume (MPV) are inexpensive, potential biomarkers of cardiovascular health.[1] Obtaining further understanding of those associations could have important clinical implications for the prevention and treatment of cardiovascular health conditions.[1] Several studies have analyzed links among cardiovascular health indicators and PLT or MPV, but these studies have often involved small samples,[2–4] covered unique populations,[5–8] or primarily analyzed only a few cardiovascular indicators at a time.[9–11] The purpose of this study was to evaluate PLT and MPV associations with a more comprehensive set of cardiovascular health indicators and outcomes in the Framingham Heart Study (FHS) in order to develop a better understanding of those interrelationships. Health indicators assessed in this study in FHS include cardiovascular disease risk factors, alcohol and prescription drug intake, and diabetes prevalence. Outcomes examined include cardiovascular disease (CVD), cerebrovascular accidents (CVA) and pulmonary embolism or deep vein thrombosis (PE/DVT).

Past research has defined PLTs of 150,000–400,000/μL as normal, being a fairly stable normally distributed reference range across populations. Lower counts indicate thrombocytopenia, and higher counts, thrombocytosis.[5] Average PLTs vary in the United States population based on age, gender, and ethnicity,[11] and previous research has suggested that PLT may be based in part on genetic inheritance.[5;12] However, lifestyle, disease, and other factors may also play a role in determining an individual's platelet count. In one study, for example, higher PLTs were associated with iron deficiency, infection, and thalassemia among hospital patients.[13] Meanwhile, low PLTs have been linked to various forms of cancer, autoimmune disease, and alcohol abuse, among others.[14]

The size of a single blood platelet is generally determined while undergoing development in the bone marrow, and mean platelet volumes provide an estimate of size within individuals.[15] MPV distributions tend to demonstrate skewness and have been shown to have more variable reference distributions across populations and different measuring equipment [16;17]. Stimuli as varied as genetics, weight, and disease states, such as ischemia, can influence MPV levels.[15] Larger platelets may contribute to the stiffening and clotting of blood vessels.[18;19] In previous research, mean platelet volume has shown positive associations with arterial stiffness,[19] atrial stasis,[20] and cardiovascular disease.[18;21]

MPV typically has a non-linear but inverse relationship with PLT, although that relationship can take other forms under certain health conditions.[22] For example, some studies have found higher MPV and lower PLT among heart disease or heart attack sufferers than among the general population,[21;23] while combined high MPV and PLT values have been linked to iron deficiencies and inflammation.[22]

The Framingham Heart Study (FHS) is a population-based study, consisting of an ongoing series of primarily family-based cohorts first developed in 1948 and based in Framingham, MA.[24] Over the last 66 years, data from the FHS have made significant contributions to cardiovascular research, including findings that weight, cholesterol, smoking, and lack of exercise affect heart disease risk. Our primary aim was to conduct a comprehensive cross-sectional analysis of PLT, MPV, and CVD-related health indicator associations in the FHS. In addition to being one of the first large population studies to primarily analyze platelet indices against a full range of cardiovascular risk factors and outcomes, this study also analyzed platelet-rich plasma platelet count (PRPPC), which is an alternate measurement of platelet count.

METHODS

Study population

This analysis was approved by an institutional review board from the National Heart, Lung, and Blood Institute (NHLBI). Data from three Framingham cohorts were used for this analysis (Original[24], Offspring[25], and Third Generation[26]). All cohorts contained individuals predominantly of European ancestry. Standard PLT counts for the Original cohort were obtained from exam 20 (1986–1990, n=1,048) through Complete Blood Count using a Baker 9000 Hematology Analyzer (Baker Instruments Corp.) from the Tufts University USDA Human Nutrition Research Center's Nutrition Education Laboratory. PRPPC was obtained from the Offspring cohort (established in 1971) at exam 5 (1991–1995, n=2,764). Blood samples in these exams were collected in evacuated tubes containing 3.7% tri-sodium citrate. Samples were centrifuged at 240 g for 10 minutes at room temperature; PRPPC counts were then measured using the Coulter Cell Counter; Model Zf (Coulter Corp.). Standard blood PLT and MPV measures were obtained from the Third Generation cohort (established in 2002) at exam 2 (2008–2011, n=2,438) through a Complete Blood Count and measured using the Coulter HmX Hematology Analyzer (Beckman Coulter, Inc.).

Individuals were excluded who were reported as having been diagnosed with leukemia or lymphoma prior to the exam, as these conditions have been linked to low blood cell counts. After these exclusions, 1,031; 2,747; and 2,427 individuals, respectively, were available for analysis. In multivariable linear regression analysis, individuals with missing covariates were excluded from analysis, bringing the number of total participants in each cohort to 964 (67 excluded), 2,699 (48 excluded), and 2,419 (8 excluded), respectively.

Independent variables

Among the remaining samples, 20 variables measured at the respective index exam cycle were selected for analysis in addition to MPV, PRPPC and PLT, including demographic factors (age, gender), biometric indicators (systolic and diastolic blood pressure taken as the average of three seated, resting measures, high-density and low-density cholesterol, triglycerides, total cholesterol, body mass index), health behaviors (smoking, alcohol intake), medication use (aspirin, diabetes medication, hypertension medication, anti-coagulant and anti-platelet treatment), health outcomes (diabetes, cardiovascular disease, cerebrovascular accidents (CVA), and pulmonary embolism or deep vein thrombosis (PE/

DVT)). These health outcomes were classified as either “prevalent” (occurring before the baseline exam in which blood platelets were ascertained) or “incident” (occurring since that time, excluding participants with previous health outcomes at the baseline exam). Body mass index (BMI) was calculated as (weight in kilograms)/(height in meters)^2. Pack-years of cigarette smoking were available in the Original and Offspring cohorts and were natural log-transformed and used in secondary analysis, as well as menopause and hormone replacement therapy use in the Offspring cohort. Tenth percentile cutpoints for MPV, PRPPC and PLT were determined in a sex-specific manner pooled across the 3 cohorts.

In the current study, cigarette smoking was defined as a self-report of having “regularly” smoked cigarettes over the past year, alcohol use as drinking at least once weekly, and aspirin use as taking at least one baby aspirin weekly. Diabetes was defined as either taking diabetes medication or as having a fasting glucose level of ≥ 126 mg/dL.[27] All medication use variables, including aspirin, diabetes medication, hypertension medication, and anticoagulant and antiplatelet treatment use, were self-reported.

Definitions of events for incidence analyses

Three categories of health outcomes were considered in incidence analyses: 1) Cardiovascular disease, 2) Cerebrovascular accidents, and 3) pulmonary embolism/deep vein thrombosis (PE/DVT). These were each defined based on standard sequence of events codes from the Framingham Heart Study, with events adjudicated by a group of physicians. Cardiovascular disease outcomes are defined to include recognized or unrecognized myocardial infarctions, angina pectoris, definite coronary insufficiency, cerebrovascular accidents (see below), sudden or non-sudden cardiac death, cerebrovascular death, intermittent claudication or congestive heart failure. Cerebrovascular accidents were categorized to include cerebrovascular accidents, atherothrombotic infarction, transient ischemic attack, cerebral embolism, and intracerebral or subarachnoid hemorrhage. Pulmonary embolism/deep vein thrombosis was classified as having had a PE or DVT diagnosis.

Variation in platelet data and variables across cohorts

Although methods of data collection among exams generally remained consistent over time across the cohorts studied, some variables were not collected at each exam. For example, PLT was only available in the Original and Third Generation cohorts, while MPV was only available in the Third generation and PRPPC was only available in the Offspring cohort. The Original cohort exam 20 did not have information on prevalent PE/DVT, LDL cholesterol, or triglyceride levels. Cigarette smoking pack-years and general antiplatelet and anticoagulant use were not available for the Third Generation.

Statistical analysis

The frequency distributions of categorical variables and the medians and ranges of continuous variables were calculated and reported. Linear regression analysis was used to analyze associations among each independent variable with MPV, PLT, or PRPPC, based on cohort. Gender interactions were also assessed for these variables. In the main analyses individual associations with each independent variable were adjusted for age and gender. To

select covariates to include in adjusted parsimonious models, full multivariable linear regression models using all 20 covariates were run by cohort for MPV, PLT and PRPPC using stepwise procedures with $p<0.05$ as the entry criteria. The effect of covariates was summarized using beta coefficients from the linear regression models. From these models, partial and full model R^2 values indicating the proportion of variability accounted for by the individual and full model, respectively, were reported. To limit type I error due to multiple testing, i.e., the chance for a significant association when none exists, a Bonferroni-adjusted significance level of 0.05/75 was used ($P<6.67\times10^{-4}$), where 75 represented the number of pre-specified separate regression models built.

Time-to-event analysis was performed in the Original and Offspring cohorts of platelet indices and the occurrence of CVD, CVA and PE/DVT after the exam. Time to event was calculated as the difference between the event date and the baseline exam date. For participants who died or were lost to follow-up, the censoring dates were the date of death or date last seen. PLT and PRPPC strata were defined as the bottom 10%, middle 80%, and top 10% for each gender. Associations with these strata were summarized using Kaplan-Meier plots and the strata were compared using the log-rank test. After confirming the validity of the Cox proportional hazards model assumption, Cox models were used to further model those nominally-significant associations based on the log-rank test by adjusting for gender and age. The effect of covariates was summarized using hazard ratios and 95% confidence intervals.

PLT and PRPPC associations with smoking were explored in simple and multivariable-adjusted Cox regression models. Individuals who had accumulated ≥0.5 pack-years of cigarette smoking during their lifetime were considered as past/current smokers. An additional set of individual regression models was also built using smoking as a three-level variable (current, former, never) rather than as a two-level variable (smoking in the last year versus not). For the Original and Offspring cohorts, “current smoking” was considered smoking in the last year. However, in the Third Generation cohort, one question was available that asked “do you smoke now?”, and that was used for current smoking variable in that cohort instead. One individual in the Original cohort was removed from the three-level variable analysis due to conflicting information relative to two-level variable analysis on current versus former smoking status.

Group means for PRPPC associations with women in the Offspring cohort were also assessed based on menopause status and hormone replacement therapy use using analysis of variance (ANOVA). Statistical analysis was conducted using the Statistical Applications Software, SAS version 9.3.

RESULTS

Demographic characteristics at the respective baseline exam for each cohort are presented in Table 1. Table 2 shows the summary of age- and gender-adjusted individual covariate regression model beta coefficients for each cohort, and it also indicates which of the variables had significant ($p<0.05$) interactions with gender. Female gender, current smoking and total cholesterol were consistently positively associated with platelet count/PRPPC

across the cohorts, although the association with former smoking was not significant. Additional associations were seen with PLT and systolic and diastolic blood pressure in the Original cohort; PRPPC, diabetes medication and systolic and diastolic blood pressure in the Offspring cohort; and with PLT and BMI in the Third Generation cohort. Platelet volume and count were inversely related in the Third Generation, and cholesterol-related indicators were negatively associated with platelet volume. Qualitatively similar results were observed when the analysis was repeated using after natural log-transformed PLT, PRPPC and MPV (data not shown).

Table 3 provides a summary and beta coefficients of the four full stepwise multivariable linear regression models. The largest coefficient of determination, R^2, was observed in the Third Generation models because both MPV and PLT were available as covariates for that cohort. In multivariable-adjusted models in each cohort, age was consistently a significant associated with PLT, PRPPC and MPV. Prevalent cerebrovascular accidents and PLT were statistically significant in the Original cohort, while aspirin use and PRPPC/MPV was statistically significant in Offspring/Third Generation cohorts, respectively.

In the Original cohort, for each unit increase in cigarette smoking pack-years, platelet count increased about 8 units after adjusting for age and gender (β=7.79, P=0.04), but that association was attenuated and became no longer significant after adjusting for all covariates. In the Offspring cohort, however, for every one unit increase in cigarette smoking pack-years, PRPPC increased by 499 units after adjustment for all covariates (β=499.40, P=0.002).

An exploratory secondary sex-specific analysis showed that associations within cohorts generally remained consistent to sex-pooled associations (Table 4). Uniformly stronger associations were seen with BMI, triglycerides, and DBP in women, while stronger associations were generally seen with aspirin in men. In the Offspring cohort menopause analysis, average platelet count was not statistically different among pre-menopausal women, women who had reached menopause and were taking hormone replacement therapy, and women who had reached menopause but were not taking hormone replacement therapy (p=0.22).

The log-rank test and Kaplan-Meier plots showed that lower platelet counts were nominally associated with higher incident CVD in the Original cohort (p=0.01), and that PRPPC was associated with incident cerebrovascular accidents in the Offspring cohort (p=0.03). The CVD Cox regression model satisfied the assumptions of proportionality of hazards test. Low platelet count remained significant in a basic Cox Proportional Hazards model adjusted for age and gender (HR for lowest 10^{th} percentile of platelet counts: 1.55, 95% CI: 1.09–2.21, p=0.02).

DISCUSSION

The mean values and distribution of variables were somewhat consistent among cohorts. Participant age and exam years probably accounted for greater differences among cohorts, such as total cholesterol, incident/prevalent disease, systolic blood pressure, BMI, and the

ratio of male to female participants ($p<0.0001$). Consistent with these trends, for example, it is known that systolic blood pressure increases with age[28] and that adult obesity levels have increased over the last few decades in the United States.[29]

Previous studies have not looked at PRPPC associations with cardiovascular health, but associations with PRPPC in the Offspring cohort were generally similar to PLT associations in other cohorts. The most consistent associations with PLT/PRPPC in individual regression analyses were found with gender, smoking and cholesterol, and these associations generally remained significant in full stepwise regression models. While associations with gender have been reported in previous literature,[7;11;30] reported associations with smoking been inconsistent,[7;9;30;31] and only a few prior studies have looked closely at cholesterol-platelet associations.[8;32]

Gender differences

In our study, female PLT and PRPPC exceeded male values (by 34×10^3/uL in the Original cohort, 23.5×10^3/uL in the Third Generation, and 861.16 in the Offspring cohort), although males and females had similar MPV (difference of 0.06 fL, $p>0.05$) (data not shown). Similar findings have been reported in the literature: female platelet counts exceeded those of men by 19×10^3/uL in a US cross-sectional study,[11] by 22×10^3/uL in an Israeli study,[7] and by 30.5×10^3/uL in a recent Polish study,[30] and in the latter, mean platelet volumes were not statistically different between genders.[30]

It has been suggested that the difference in PLT values between men and women may exist in part due to the presence of estrogen, which has been shown to play a role in encouraging platelet production.[11;33] However, in our Offspring menopause analysis, PRPPC did not vary in women based on menopause status and use of hormone replacement therapy. In two other studies, gender-based platelet count differences remained significant in old age[11;34]. Furthermore, functional studies in mice indicate little effect of estrogen on PLT.[35]

In our study, BMI, DBP and triglycerides generally showed an association with platelet count/volume in women but not in men (Table 4). Previous literature has suggested that adipose tissue may promote platelet development by stimulating chronic inflammation.[36] This possibility may be of particular relevance for women, who generally have higher body fat levels than men with the same BMI, and other research has shown an association of platelet count and several obesity-related indicators specifically in women.[6;36;37] Expression levels of specific platelet transcripts have also been linked to BMI.[38]

Cigarette smoking

In our three cohorts, cigarette smoking within the past year showed a consistent association with higher platelet count both overall and for men and women separately. Our secondary analysis confirmed that finding using pack-years as an indicator. Former smoking was not significant in association analyses. Together these findings suggest that as smokers quit or reduce smoking levels effects on platelets likely attenuate. Our findings shed new light on conflicting results regarding platelet counts and smoking in the literature. In one previous study, females who smoked had statistically significant lower platelet counts than those who did not smoke, while the association with men was non-significant.[7] On the other hand, a

Nigerian study found that male smokers had a higher platelet count than male non-smokers,[9] and a recent rat study showed a positive association between smoking exposure and platelet count.[31] The difference among these studies may be explained in part by a variety of confounding factors, such as study design, participant age, sample sizes, and geography. Our finding that platelet count and smoking status are positively associated could be biologically plausible, as previous literature has discussed potential associations among smoking, vascular inflammation, and blood clotting in men,[39] but more research is warranted to better understand those connections.

Cholesterol

Our study found associations between cholesterol indicators and platelet count indices and a negative association with platelet volume. Similarly, a recent Japanese study showed that platelet count had a positive association with the LDL/HDL cholesterol ratio and a negative association with HDL cholesterol as well as positive associations with aggregability.[40] However, another study found that platelet count increased with both HDL and LDL cholesterol levels and did not see a significant association with MPV,[8] so these relationships should be further explored. The association between cholesterol levels and platelet indices may be related to inflammation, as discussed for BMI and smoking, as research has found a positive link between platelet count and C-reactive protein levels[8] as well as platelet transcript expression and C-reactive protein or interleukin 6 levels.[41]

Additional findings

Associations were seen with diabetes medication in the Offspring cohort, BMI in the Third Generation cohort, and with blood pressure in the Offspring and Original cohorts. These associations may all be secondary indicators of previously discussed variables, although the diabetes finding was particularly interesting because it showed a negative association with platelet count. In other research, platelet volume has been reported to be positively associated with diabetes status.[2] Our Third Generation results seem consistent with this, though they do not remain significant after Bonferroni correction.

After adjustment for age and gender, lower platelet count was associated with incident CVD. In recent research, patients with myocardial infarction or unstable angina had higher platelet volume and lower platelet count than controls.[23] Participants in the Offspring cohort who later had one of these diseases may have had higher MPV levels as well, but as those data were not collected, this hypothesis is merely speculative.

While small positive associations were seen with aspirin and platelet indices, it is possible that its association with aspirin use was tied to other covariates, such as weight and blood pressure. In a previous clinical trial, platelet count and total platelet mass increased over a seven-day period among middle-aged males taking aspirin.[42] Still, examination of additional randomized trials would be warranted to better understand these reported associations.

Limitations

In this study, not all variables were available for each cohort, and some variable definitions changed over time. For this reason, we did not conduct cohort-pooled analyses, which could

have improved the statistical power. It should also be noted that PRPPC and PLT were generally discussed together even though they represent different platelet counting techniques. We assumed that PRPPC differed from whole blood PLT by a factor related to centrifugation, and that if samples were treated similarly, PRPPC would be reflective of PLT. Recent or longer-term alterations in dietary intake and physical activity may influence blood measures [43;44]. Participants in the current study were fasting reducing the likely impact of recent dietary intake. In this study we had no means to assess recent physical activity though most individuals were considered likely to be sedentary[45]. The current study is biased toward population of European ancestry so some of the findings may have limited generalizability to other groups of non-European ancestry.

Conclusions

This study shows that platelet count is positively associated with female gender, as well as total cholesterol and cigarette smoking. Future studies may examine replication in other cohorts with differing ethnic makeups in order to better understand the relationship among these variables and platelet indices, as well as examining longitudinal and cross-sectional associations with clinical events in larger samples.

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Abbreviations

BMI	Body mass index
CVD	Cardiovascular disease
CVA	Cerebrovascular accidents
DBP	Diastolic blood pressure
FHS	Framingham Heart Study
HDL	High density lipoprotein
LDL	Low density lipoprotein
MPV	mean platelet volume
NHLBI	National Heart, Lung, and Blood Institute
PLT	platelet count
PRPPC	platelet-rich plasma platelet count
PE/DVT	pulmonary embolism or deep vein thrombosis
SBP	Systolic blood pressure

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Table 1

Descriptive baseline characteristics by cohort.

	Original Exam 20 (n=1,031) 1986–1992		Offspring Exam 5 (n=2,747) 1991–1995		Third Generation Exam 2 (n=2,427) 2008–2011	
Binary variables	**Number**	**%**	**Number**	**%**	**Number**	**%**
Gender (male)	404	39.19	1,288	46.89	1,191	49.07
Hypertension medication	564	54.70	553	20.13	461	19
Cigarette smoking in the last year	106	10.29	511	18.6	288	11.87
Current smoker (do you smoke now?)	--	--	--	--	258	10.63
Former smoker	451	43.83	1,324	48.23	35	1.44
Alcohol	516	50.34	1,894	68.95	1,938	79.85
Aspirin	327	33.44	716	26.22	375	15.46
Diabetes	123	11.93	203	7.39	165	6.8
Diabetes treatment	76	7.38	93	3.39	99	4.08
PE/DVT (prevalence)	---		6	0.22	20	0.82
PE/DVT (incidence)	52	5.04	116	4.22	4	0.16
CVD (prevalence)	340	32.98	279	10.16	65	2.68
CVD (incidence)	348	33.75	498	18.13	15	0.62
CVA (prevalence)	104	10.09	45	1.64	19	0.78
CVA (incidence)	178	17.26	212	7.72	4	0.16
Antiplatelet use	36	3.51	10	0.36	---	
Anticoagulant use	26	2.54	26	0.95	---	
Discrete/continuous variables	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age, years	76.24	5.85	55.27	9.94	47.22	8.73
PLT ($\times 10^3$/uL)	256.52	76.02	---		246.62	56.24
MPV (fL)	---		---		8.50	0.92
PRPPC	---		27,854.00	6,403.59	---	
SBP (mmHg)	146.58	22.47	126.60	19.43	117.22	14.31
DBP (mmHg)	75.47	11.42	74.31	10.15	74.98	9.22
HDL (mg/dL)	48.07	15.41	50.00	15.38	59.63	18.20

	Original Exam 20 (n=1,031) 1986–1992		Offspring Exam 5 (n=2,747) 1991–1995		Third Generation Exam 2 (n=2,427) 2008–2011	
Binary variables	**Number**	**%**	**Number**	**%**	**Number**	**%**
LDL (mg/dL)	---		126.80	32.71	104.57	31.32
Triglycerides (mg/dL)	---		149.78	116.98	115.51	78.54
Total cholesterol (mg/dL)	213.53	41.77	205.21	36.64	187.19	34.63
BMI	26.53	4.67	27.44	5.06	28.26	5.87
Smoking (pack-years)	15.25	22.44	17.50	23.13	---	

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Table 2

Results of individual regression models by cohort, each adjusted for age and gender.

	Original cohort (PLT)		Offspring (PRPPC)		Third Generation (PLT)		Third Generation (MPV)	
Variable	**Parameter**	**P-value**	**Parameter**	**P-value**	**Parameter**	**P-value**	**Parameter**	**P-value**
Gender (female)[a]	**30.88**[b]	**<.0001**	825.82	0.0008	**23.35**	**<.0001**	0.0687	0.0657
Age, years[c]	0.38	0.3574	−37.24	0.0026	−0.19	0.1477*	−0.0044	0.041
Hypertension medication	−2.93	0.5435	269.39	0.4049	10.06	0.0008*	−0.0094	0.851
Smoked cigarettes regularly (last year)	**34.90**	**<.0001**	**1,377.61**	**<.0001**	**14.83**	**<.0001**	−0.1147	0.0474
Current smoker[d]	**35.28**	**<.0001**	**1,338.98**	**0.0002**	**15.35**	**<.0001**	−0.1300	0.0278
Former smoker[d]	6.96	.1617	81.16	0.7668	11.17	0.2332	−0.0900	0.5462
Pack years (ln)	7.79	0.0403	**574.98**	**<.0001**	---		---	
Alcohol use	1.54	0.755	31.95	0.9052*	−3.09	0.2708	−0.0312	0.5042
Aspirin use	1.09	0.8309*	476.81	0.0962	0.35	0.9172	0.1287	0.0204
Diabetes (prevalence)	1.85	0.8043	−1,479.86	0.0023	4.97	0.2722*	0.1572	0.0369
Diabetes medication	−11.82	0.1987	**−2,498.73**	**0.0003**	12.39	0.0297	0.0928	0.3284
PE/DVT (prevalence)	---[e]		1,043.95	0.6891	35.22	0.0045	−0.0837	0.685
CVD (prevalence)	−3.67	0.4797	−383.07	0.3675	1.25	0.8588**	0.0686	0.5579
CVA (prevalence)	−13.01	0.0984	−1,171.41	0.231	−10.88	0.3916	0.0238	0.9104
SBP (mmHg)	0.06	0.6069	22.55	0.0011*	**0.37**	**<.0001***	0.0004	0.7866
DBP (mmHg)	−0.19	0.3895	**49.74**	**<.0001**	**0.64**	**<.0001***	0.0004	0.8458
HDL (mg/dL)	0.30	0.0721	−1.75	0.8441*	−0.16	0.0235**	−0.0008	0.4884

	Original cohort (PLT)		Offspring (PRPPC)		Third Generation (PLT)		Third Generation (MPV)	
Variable	**Parameter**	**P-value**	**Parameter**	**P-value**	**Parameter**	**P-value**	**Parameter**	**P-value**
LDL (mg/dL)	---		**22.26**	**<.0001**	**0.28**	**<.0001**	−0.0016	0.0084
Triglycerides (mg/dL)	---		**4.42**	**0.0003**	**0.09**	**<.0001**	−0.0003	0.0265
Total cholesterol (mg/dL)	**0.23**	**0.0001**	**25.33**	**<.0001**	**0.26**	**<.0001*****	**−0.0020**	**0.0003**
PLT($\times 10^3$/uL)	---		---		---		**−0.0069**	**<.0001**
MPV (fL)	---		---		**−25.04**	**<.0001**	---	
BMI	−0.06	0.902	34.65	0.1592	**1.03**	**<.0001****	0.0053	0.1033
Antiplatelets	−9.86	0.4558	−1,335.37	0.5097	---		---	
Anticoagulants	4.56	0.7678	−1,573.50	0.2122	---		---	

[a] Adjusted for age only,

[b] Values in bold are significant after Bonferroni correction ($p<6.67\times10^{-4}$),

[c] Adjusted for gender only,

[d] "Current" and "former" smoking were part of a three-level variable in an individual regression model, with "never" smoker considered the referent value. See "Methods" section for more.

[e] "---" indicates PE/DVT, LDL cholesterol, triglycerides, PLT, MPV, pack years, or antiplatelets and anticoagulants were unavailable for these exams. See "Methods" section for more.

*,**,*** indicates significant interaction term with gender (*$0.01\leq p<0.05$, **$0.001\leq p<0.01$, ***$p<0.001$)

Table 3

Summary of statistically significant results of stepwise regression models

	Original cohort (PLT)		Offspring (PRPPC)		Third Generation (PLT)		Third Generation (MPV)	
Variable	**Partial R^2**	**P-value**	**Partial R^2**	**P-value**	**Partial R^2**	**P-value**	**Partial R^2**	**P-value**
Gender (female)	0.041	<.0001	0.003	0.0041	0.050	<0.0001	0.015	<0.0001
Age, years	0.005	0.0249	0.008	<0.0001	0.005	0.0001	0.004	0.0006
Hypertension medication					0.002	0.0170		
Cigarette smoking	0.018	<.0001	0.006	<0.0001	0.004	0.0003		
Aspirin use			0.002	0.0378			0.002	0.0309
Diabetes medication			0.004	0.0005	0.003	0.0038		
PE/DVT (prevalence)					0.002	0.0071		
CVA (prevalence)	0.004	0.0350						
DBP (mmHg)			0.003	0.0068	0.004	0.0003		
LDL cholesterol (mg/dL)					0.016	<0.0001		
Total cholesterol (mg/dL)	0.012	0.0005	0.017	<0.0001				
MPV (fL)					0.158	<0.0001		
PLT ($\times 10^3$/uL)							0.158	<0.0001
BMI					0.011	<0.0001	0.005	0.0001
Full model R^2	*0.080*		*0.050*		*0.256*		*0.187*	

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Table 4

Statistically significant P-values from multivariable regression models in each cohort by gender

	Original		Offspring		Third Generation (PLT)		Third Generation (MPV)	
Variables	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**
Age, years		0.0430	*0.0004*[a]	*0.0244*	*0.0007*	*0.0079*	*0.0096*	*0.0295*
Hypertension medication						0.0055		
Cigarette smoking	<0.0001	0.0279	0.0161	0.0004	0.0001			
Aspirin use	0.0276				0.0267		0.0041	
Diabetes			*0.0116*					
Diabetes medication				*0.0043*				
PE/DVT (prevalence)					0.0014			
DBP (mmHg)		*0.0265*		0.0023		0.0071		
HDL cholesterol(mg/dL)								
LDL cholesterol(mg/dL)						<0.0001		
Triglycerides(mg/dL)				0.0144		<0.0001		
Total cholesterol(mg/dL)	0.0117	0.0062	<0.0001	<0.0001	0.0081			
PLT($\times 10^3$/uL)							*<0.0001*	*<0.0001*
MPV (fL)					*<0.0001*	*<0.0001*		
BMI						<0.0001		<0.0001

[a] Variables with negative associations with platelet indices in the final step of the stepwise regression model have P-values shown in italics.