Polygenic Risk Scores for Cardiovascular Diseases Influence Different Platelet Reactivity Tests

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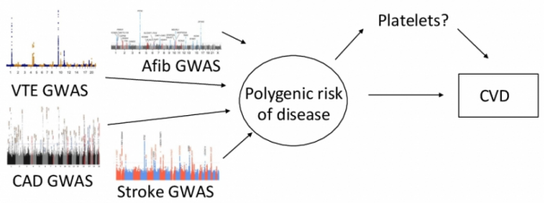
Introduction

Genome-wide association studies (GWAS) revolutionized human genetics by identifying DNA variations, or single nucleotide polymorphisms (SNPs), common to specific traits and diseases. GWAS results can be used to derive polygenic risk scores (PRS), which assess the genetic risk of disease in an individual through weighting and summing an ensemble of SNPs. Given the complex and multifactorial nature of cardiovascular disease (CVD), PRS have been derived in the past with a view to identify individuals at higher risk for CVD and may ultimately have clinical utility in addition to traditional risk factors. Platelets, being key mediators of thrombosis and hemostasis, have also been studied as biomarkers of CVD.

Objective

Analyze the association of individuals' PRS for multiple CVD outcomes with platelet aggregation trait values derived from five distinct assays. We hypothesize that those with higher CVD PRS will have increased platelet aggregation trait values.

Figure 1. Through association testing between PRS and platelet reactivity traits, we hope to establish platelets as a mediator between genetics and CVD.



Methods

PRS Derivation: We first collected the largest available polygenic risk scores for coronary artery disease (CAD), atrial fibrillation (AF), stroke, and venous thromboembolism (VTE). Each study varied in the number of variants, cases, and controls (**Table 1**).

CVD PRS	Cases	Controls	Variants Included	Method	Parameters
Stroke	40,585	406,111	82	p+t	$P < 1x10^{-5}$ $R^2 < 0.05$
VTE	23,151	553,439	267	p+t	$p < 1x10^{-5}$ $R^2 < 0.02$
CAD	60,801	123,504	6,084,629	LDPred	ρ=0.001
AF	17,931	115,142	6,098,901	LDPred	ρ=0.003

Table 1. Overview of CVD PRS used in our study

Follow-up VTE SNP Analysis: Following our primary analysis, we tested associations between the 267 VTE PRS SNPs and the four strongest platelet reactivity traits (T-TAS AUC, MP collagen AUC, MP ristocetin AUC, and LTA ristocetin primary slope)

Platelet Reactivity: Platelet reactivity traits (134) were assessed in the Framingham Heart Study (FHS) Generation 3 and New Offspring Spouse cohorts via 5 distinct assays and a range of platelet agonists: light transmission aggregometry (LTA), Multiplate impedance aggregometry (MP), Total-Thrombus Formation Analysis System (T-TAS), Optimul 96-well plate assay, and ADP-stimulated flow cytometry (FC).

Statistical Threshold: Principal component analysis revealed 45 of our 134 total traits to independently explain 90% of result variation. Thus, we corrected for multiple trait testing in our four CVD indicators via Bonferonni correction (0.5/45*4) and obtained a statistical significance threshold of P<2.78E-4.

Statistical Analysis: For each platelet trait, we used linear regression adjusting for age and sex to derive residuals and applied inverse normal transformation to the residuals; we then tested association between each PRS adjusting for aspirin use in the overall sample.

Results

PRS Distribution and Validation: Polygenic risk scores for each CVD followed an expected normal distribution in the FHS; PRS were significantly associated with each CVD in a pooled cohort of Generation 2, Generation 3, and New Offspring Spouse participants in the FHS.

VTE PRS and Platelet Reactivity Traits: VTE PRS analysis revealed two significant positive associations with the platelet aggregation traits T-TAS AUC and LTA ristocetin primary slope. Consistent with this, we observed numerous trends just below statistical significance that implicated those with a higher PRS also had more reactive platelets. **Table 2** shows our top 5 strongest traits with VTE PRS.

AF, CAD, Stroke PRS and Platelet Reactivity Traits: While no traits reached statistical significance in AF, CAD, or stroke PRS analyses, we observed a common trend of higher ADP-stimulated platelet activation markers in FC experiments with these PRS.

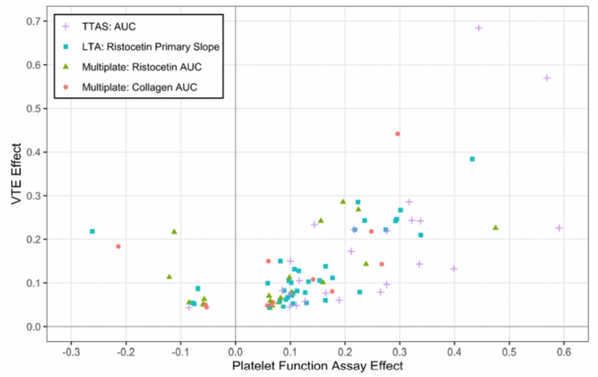
Table 2. Top 5 strongest associations between VTE PRS and platelet reactivity traits. Top 2 traits passed our statistical significance threshold. LTA indicates light transmission aggregometry; TTAS indicates Total Thrombus Formation Analysis System; FC indicates flow cytometry.

Main sample		Age-sex-aspirin adjusted			
Trait	Assay Type	Beta	SE	P-value	N
Ristocetin primary slope	LTA	0.0961	0.0190	4.52E-07	2852
TTAS AUC	TTAS	0.1112	0.0282	8.22E-05	982
WB normal saline estimated platelet count	FC	0.0735	0.0207	3.74E-04	2479
Ristocetin AUC	Multiplate	0.0544	0.0174	1.83E-03	2922
Collagen AUC	Multiplate	0.0566	0.0182	1.83E-03	2957

Results

VTE SNP Analysis: Of the 267 tests for each of the four traits, we observed 161 (60.3%) T-TAS AUC tests, 160 (59.9%) MP ristocetin AUC tests, 153 (57.3%) MP collagen AUC tests, and 168 (62.9%) LTA ristocetin primary slope tests to have an effect direction where the platelet reactivity increasing allele was also the VTE risk increasing allele, suggesting that the VTE PRS platelet trait associations were likely not driven by single SNPs or loci

Figure 2. Directionality of 267 independent SNPs used in VTE PRS and four strongest platelet trait associations.



Discussion

Strong associations between multiple platelet assays and VTE PRS suggest distinct platelet genes implicated in this disease pathophysiology. Given an expanding set of anti-platelet drugs (e.g., GP6, GP1b, PAR4 inhibitors) in development, targeting specific CVD indications could be suggested based on the platelet pathways implicated by our results.

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