Exome sequencing from extreme responders to aspirin identifies a novel variant associated with platelet aggregation

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Introduction

- Platelet dysregulation is integral in the formation of thrombi, and abnormal thrombus formation may ultimately lead to myocardial infarction or stroke.
- Platelets, and more specifically platelet activation and aggregation pathways, are prime targets for therapeutic intervention.
- Aspirin or Dual Anti-Platelet Therapy with aspirin and clopidogrel (DAPT) is often prescribed for primary and secondary prevention of cardiovascular events.
- A high degree of inter-individual variability exists, resulting in sub-optimal therapy for many patients.

Objectives

- **To identify novel variants that may significantly impact platelet response to aspirin and DAPT.**

Methods

HAPI and PAPI Population

- Old Order Amish of Lancaster, PA.
- Founder population of Swiss origin that immigrated during the 18th century.
- Extensive family history records.
- HAPI study: 851 healthy subjects on a two-week 81mg/day aspirin intervention.
- PAPI study: 661 healthy subjects received 1 week of clopidogrel therapy (75mg/day) with one 81mg dose of aspirin on the last day of treatment.

Sinai Hospital Cohort

- The Sinai Hospital cohort is composed of 350 non-emergent PCI patients on clopidogrel and aspirin.

Study Measures

- Platelet function was measured by optical aggregometry with a PAP8E aggregometer (Bio/Data Corporation, Horsham, Pennsylvania) for HAPI and PAPI and a Chronolog Lumi-Aggregometer (Model 490-4D; Chronolog, Havertown, Pennsylvania, USA) for Sinai stimulated with Collagen (2 µg/ml) and was expressed as the maximal percentage change in light transmittance. HAPI was conducted in whole blood. PAPI and Sinai were conducted in platelet-rich plasma.

Sequencing and Genotyping

- Exome sequencing was conducted on an ABI Solid sequencing platform in 30 HAPI subjects.
- Taqman genotyping was conducted for the full HAPI, PAPI, and Sinai cohorts..

Analysis

- Case-control analysis was performed using Plinkseq with 30 HAPI subjects from the extremes of collagen-mediated platelet aggregation response to aspirin.
- For HAPI and PAPI, multivariate regression analyses adjusted for relatedness, age, sex, bmi, and baseline platelet aggregation was conducted using our mixed models analysis for pedigrees and populations (MMAP) software http://edn.som.umaryland.edu /mmap/index.php.
- For Sinai, regression analysis adjusted for age, sex, BMI, ethnicity, and study was conducted using SAS 9.2.

Results

Case-Control Analysis:

- Most significant variants are identified in Table 1.
- Cases defined as high aspirin responders and controls defined as low responders to aspirin.
- A synonymous variant (rs17834991, G allele MAF=0.16) in the *SVIL* gene was enriched in high responders ($p=6.5x10^{-3}$).

Follow-up Genotyping:

- rs17834991 was significantly associated with post-aspirin collagen-mediated aggregation in HAPI using whole blood (2 µg/ml collagen, beta=-0.65, $p=3.57x10^{-2}$, n=710).

Replication Analysis:

- rs17834991 was significantly associated in PAPI with post-DAPT collagen-mediated aggregation in platelet-rich plasma (2 µg/ml collagen, beta=-1.82, $p=4.98x10^{-3}$, n=661) at multiple agonist concentrations (Figure 1).
- rs17834991 was not significantly associated with post-DAPT collagen-mediated aggregation in the Sinai cohort (2 µg/ml collagen, beta=-4.235, p=0.18, n=169).

Table 1: Case-Control Analysis

SNP	Gene	MAF	WT cases	Het cases	Hom cases	WT controls	Het controls	Hom controls	P	OR
rs1652804	TSPAN15	0.51	1	4	8	8	7	2	7.75E-04	6.96
rs6897513	SPEF2	0.33	11	1	1	6	5	6	2.21E-03	0.13
rs1799801	ERCC4	0.33	10	3	0	3	11	3	2.21E-03	0.13
rs550404	SYTL2	0.61	5	6	2	1	5	11	2.78E-03	0.16
rs41289934	FAM26E	0.16	13	0	0	9	6	2	3.22E-03	0.04
rs3848519	FECH	0.1	8	4	1	17	0	0	4.60E-03	21.87
rs33935215	TRIM24	0.1	7	6	0	17	0	0	4.60E-03	21.87
rs17834991	SVIL	0.18	4	9	0	15	2	0	6.50E-03	8.47
rs2393592	HIST1H4H	0.4	9	3	1	4	7	6	7.24E-03	0.18
rs2275613	CNN3	0.25	11	2	0	5	11	1	7.59E-03	0.13

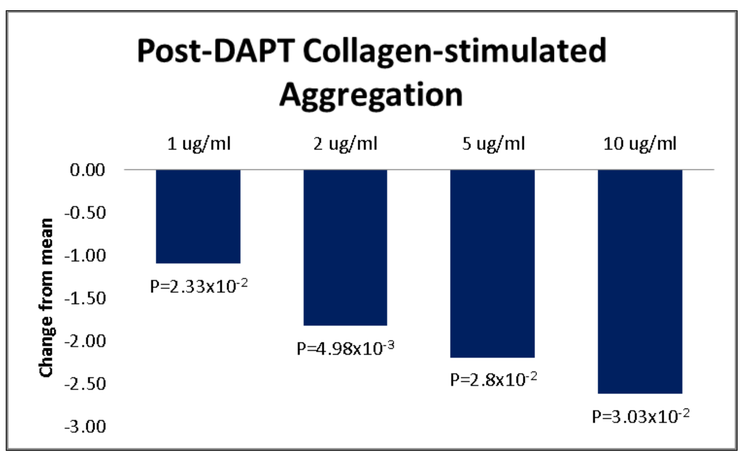

Figure 1: Platelet Aggregation Post-DAPT in PAPI study with multiple concentrations of collagen in PRP.

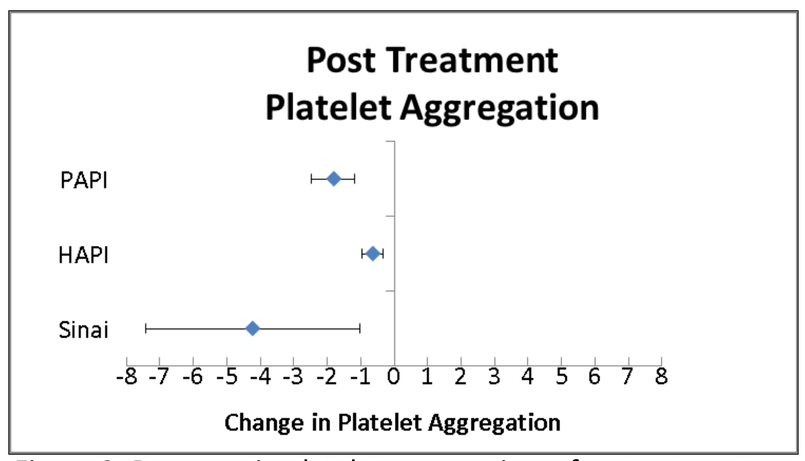

Figure 2: Decrease in platelet aggregation after treatment with rs17834991 genotype in all studies.

Discussion

- The SVIL gene encodes two proteins: a muscle-specific isoform, Archvillin, as well as a ubiquitous form, Supervillin, that is expressed in platelets.
- In a previous study, mutations in SVIL, but not rs17834991, were found to be associated with inhibition of platelet aggregation under shear stress conditions [1].
- Despite the Sinai sample not being associated , all three studies demonstrate a similar trend (see Figure 2).
- Our findings suggest that SVIL variant rs17834991 significantly alters platelet response following Aspirin and DAPT treatment.
- Taken with previous findings, SVIL may not only play a role in platelet function but may also impact anti-platelet intervention.
- Further studies are warranted to elucidate the potential impact of mutations in *SVIL* on cardiovascular disease.

References & Acknowledgements

1. Edelstein LC, et al. Circulation. 2012;125:2762-2771.

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