Human Genome-Wide Association and Mouse Knockout Approaches Identify Platelet Supervillin as an Inhibitor of Thrombus Formation Under Shear Stress

Leonard C. Edelstein, PhD; Elizabeth J. Luna, PhD; Ian B. Gibson, BA; Molly Bray, PhD; Ying Jin, MD; Altaf Kondkar, PhD; Srikanth Nagalla, MD; Nacima Hadjout-Rabi, PhD; Tara C. Smith, MS; Daniel Covarrubias, PhD; Stephen N. Jones, PhD; Firdos Ahmad, PhD; Moritz Stolla, MD; Xianguo Kong, MD; Zhiyou Fang, PhD; Wolfgang Bergmeier, PhD; Chad Shaw, PhD; Suzanne M. Leal, PhD; Paul F. Bray, MD

Background—High shear force critically regulates platelet adhesion and thrombus formation during ischemic vascular events. To identify genetic factors that influence platelet thrombus formation under high shear stress, we performed a genome-wide association study and confirmatory experiments in human and animal platelets.

Methods and Results—Closure times in the shear-dependent platelet function analyzer (PFA)–100 were measured on healthy, nondiabetic European Americans (n=125) and blacks (n=116). A genome-wide association ($P<5\times10^{-8}$) was identified with 2 single-nucleotide polymorphisms within the *SVIL* gene (chromosome 10p11.23) in African Americans but not European Americans. Microarray analyses of human platelet RNA demonstrated the presence of *SVIL* isoform 1 (supervillin) but not muscle-specific isoforms 2 and 3 (archvillin, SmAV). *SVIL* mRNA levels were associated with *SVIL* genotypes ($P\leq0.02$) and were inversely correlated with PFA-100 closure times ($P<0.04$) and platelet volume ($P<0.02$). Leukocyte-depleted platelets contained abundant levels of the ≈205-kDa supervillin polypeptide. To assess functionality, mice lacking platelet supervillin were generated and back-crossed onto a C57BL/6 background. Compared with controls, murine platelets lacking supervillin were larger by flow cytometry and confocal microscopy and exhibited enhanced platelet thrombus formation under high-shear but not low-shear conditions.

Conclusions—We show for the first time that (1) platelets contain supervillin; (2) platelet thrombus formation in the PFA-100 is associated with human *SVIL* variants and low *SVIL* expression; and (3) murine platelets lacking supervillin exhibit enhanced platelet thrombus formation at high shear stress. These data are consistent with an inhibitory role for supervillin in platelet adhesion and arterial thrombosis. **(*Circulation*. 2012;125:2762-2771.)**

Key Words: genetics ■ genome-wide association study ■ murine model ■ platelets ■ thrombosis

Arterial thrombosis is a major cause of myocardial infarction (MI) and stroke. Most clinical events occur when an atherosclerotic plaque ruptures to expose subendothelial collagen and von Willebrand factor (VWF). These proteins bind to platelets, triggering primary activation and granule secretion.[1] Secretion of soluble agonists, including ADP, amplifies activation and leads to integrin activation, platelet-platelet aggregation, and occlusive thrombus formation. Platelets play a more prominent role in arterial thrombus formation than in venous thrombosis because the highly specialized initial platelet-VWF interaction is enhanced by shear stress, such as occurs in coronary arteries.[2,3]

Clinical Perspective on p 2771

Platelet reactivity varies greatly among individuals. This variation exhibits strong heritability in both European Americans (EAs) and African Americans (AAs),[4] which could explain some of the known genetic contribution to the risk of acute MI.[5] Although genetic epidemiology screens have identified loci associated with MI risk,[6] our understanding of

Continuing medical education (CME) credit is available for this article. Go to http://cme.ahajournals.org to take the quiz.

Received January 13, 2012; accepted April 20, 2012.

From the Cardeza Foundation for Hematologic Research, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA (L.C.E., Y.J., A.K., S.N., F.A., M.S., X.K., P.F.B.); Department of Cell Biology, University of Massachusetts, Worcester (E.J.L., N.H.-R., T.C.S., S.N.J., Z.F.); Department of Genetics, Baylor College of Medicine, Houston, TX (I.B.G., D.C., C.S., S.M.L.); Departments of Epidemiology and Genetics, University of Alabama at Birmingham (M.B.); Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill (W.B.); and Department of Statistics, Rice University, Houston, TX (C.S., S.M.L.).

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.112.091462/-/DC1.

Correspondence to Paul F. Bray, MD, Cardeza Foundation for Hematologic Research, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Curtis Building, Room 324, 1015 Walnut St, Philadelphia, PA 19107. E-mail paul.bray@jefferson.edu

Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.112.091462**

causative genes is limited. The use of intermediate phenotypes to identify genes involved in the pathophysiology of arterial thrombosis can yield stronger genetic associations.[7] Genome-wide association studies (GWAS) have led to the discovery of key molecules regulating human disease[8] and have identified genetic variants and novel genes associated with platelet number, platelet volume, and in vitro platelet aggregation.[9,10] However, no GWAS has identified genes associated with shear stress–dependent platelet function. The platelet function analyzer–100 (PFA-100) measures the time to platelet thrombus formation under a defined shear stress of 1500 s^{-1} in whole blood.[11] The assay requires platelet tethering to VWF, firm adhesion to collagen, platelet activation and secretion, and platelet aggregation mediated by VWF and fibrinogen. Abnormal assay results correlate with platelet hyperfunction and hypofunction associated with acute coronary syndromes[12–15] and bleeding disorders, respectively.[16,17] The aim of this study was to identify genetic factors that influence platelet reactivity and thrombus formation under high shear stress. We performed a genome-wide screen with the PFA-100 to identify novel gene variants in AAs and EAs. We identified a novel platelet gene, *SVIL* (encoding the cytoskeletal regulatory protein supervillin), whose expression negatively regulates platelet reactivity and thrombus formation in both humans and mice.

Methods

Subjects and Platelet Phenotyping

The Platelet Genes and Physiology Study was approved by the institutional review boards of Baylor College of Medicine and Thomas Jefferson University, and informed consent was obtained from all volunteers. Healthy donors were recruited between 2000 and 2006 in Houston, TX. Citrated whole blood was used to measure PFA-100 closure times in a collagen- and ADP-impregnated cartridge (hereafter referred to as PFA-100ColA) within 30 minutes of phlebotomy. A platelet aggregation response of <10% was considered as exposure to antiplatelet agents and reason for exclusion.

Genotyping

Genomic DNA was extracted from leukocyte buffy coats with the Qiagen DNA extraction kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA from AAs was genotyped on the Illumina Hum1M Beadarray. EA DNAs were genotyped on the Illumina Hum550k Beadarray. Because of the lower levels of linkage disequilibrium in African populations, a denser single-nucleotide polymorphism (SNP) microarray was selected to genotype the DNA samples from AA individuals to improve tagging of causal variants.

Statistical Analysis

Quality control was performed before statistical analysis. Subjects were excluded for relatedness to other participants and for failing stringent genotyping quality control. Individual genotypes that failed quality control were also removed (see Methods in the online-only Data Supplement for details). PFA-100ColA closure times were natural log transformed and tested for associations with the use of an additive model within a linear regression framework. All analyses of EA and AA samples were performed separately. The potential confounders age, sex, VWF activity, plasma fibrinogen level, and platelet CD41 level were tested for significance with the use of forward selection, and significant covariates were retained within the model ($P<0.1$). Principal components analysis was also performed on the data with a subset of the SNP markers that are in linkage equilibrium ($r^2<0.3$).[18] To evaluate whether there is inflation of the test statistic due to population substructure/admixture, λ was estimated both when no principal components analysis components and when 1 through 5 principal components analysis components were included in the linear regression model.

Platelet Gene Expression Analysis

RNA from leukocyte-depleted platelets (LDP) was prepared for gene expression profiling in 29 healthy subjects. LDP was prepared with the use of density centrifugation followed by CD45-positive cell depletion of platelet-rich plasma (PRP).[19] As controls, RNA from PRP and from the $CD45^+$ leukocyte fraction was extracted from 11 and 5 subjects, respectively, with the use of TRIzol (Invitrogen, Carlsbad, CA). Gene expression analysis was performed with the Sentrix BeadChip and BeadStation system from Illumina, Inc (San Diego, CA).[20]

Platelet Supervillin Expression and Correlation With PFA-100ColA

LDP RNA was used to validate the *SVIL* microarray data.[19] Total LDP RNA was reverse transcribed and polymerase chain reaction (PCR) amplified with the use of a sense primer in coding exon 1 and an antisense primer in coding exon 5 of SVIL isoform 1. Immunoblotting of 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels was performed with 4 different anti-supervillin antibodies to verify platelet expression. Natural log–transformed mRNA expression data from microarrays were plotted versus PFA-100ColA closure times for the 23 individuals for whom both values were available. Pearson correlation r and P values were calculated with the use of GraphPad Prism software (La Jolla, CA).

Mouse Platelet Phenotyping

Mice lacking *Svil* were generated and back-crossed 10 times onto a C57BL/6 background. $Svil^{-/-}$ and control C57BL/6 mice were maintained by homozygous breeding. Platelet thrombus formation under shear stress was measured with the use of microfluidic flow chambers with immobilized collagen.[21] Blood from 3 wild-type and 3 $Svil^{-/-}$ mice was studied. Experiments were performed over 4 different days, with 3 to 4 runs per day (a "run" was defined as data acquisition from platelet deposition in a single flow chamber) for a total of 15 runs per wild-type genotype and 15 runs per $Svil^{-/-}$ genotype. P values were computed with a 2-way repeated-measures, linear mixed-effects ANOVA model that accounts for the main effect of genotype using GraphPad Prism.

Flow Cytometry

Whole blood was diluted into Tyrode's buffer containing 1 mmol/L $CaCl_2$ and stained with fluorescein isothiocyanate-α-CD41 (BD Pharmingen) or phycoerythrin-α–glycoprotein (GP)Ibα (Emfret Analytics, Würzburg, Germany). After dilution with phosphate-buffered saline, the cells were analyzed on a FACScan.

Immunofluorescence Confocal Microscopy

Platelets adhered to glass slides statically or to collagen-coated coverslips statically or under high shear stress were stained with Alexa-568 phalloidin and α-myosin IIA antibodies and imaged with confocal microscopy. Additional details are available in the online-only Data Supplement.

Results

SNPs Within SVIL Are Associated With Platelet Function Under Shear Stress

To assess the capacity for platelet thrombus formation under shear stress, PFA-100ColA was used to measure PFA-100 closure times in a cohort of healthy, nondiabetic subjects (Platelet Genes and Physiology Study). For this genetic study, only subjects self-identified as EA or AA were considered. Blood was collected for PFA-100ColA testing on 154 AAs

Table 1. Demographics of Subjects in Genetic Study

Parameter	African Americans	European Americans
No. of subjects	116	125
Women, %	69	50
Age, mean±SD, y	35.0±9.4	35.0±11.1
Body mass index, mean±SD, kg/m^2	28.6±5.3	25.5±4.6
Smokers, %	16.4	32
Hypertension, %	8.6	3.4
Hematocrit, mean±SD, %	36.1±5.0	37.4±4.4
Fibrinogen, mean±SD, mg/dL	345±99	300±64
Von Willebrand factor activity, mean±SD, %	88±38	79.7±35
Platelet-rich plasma platelet count (per μL), mean±SD	406 060±93 350	430 550±111 600

and 157 EAs. The PFA-100ColA closure time data were normally distributed in both groups (not shown). The DNA from each subject was genotyped for 620 901 or 1 070 000 tag SNPs for EAs or AAs, respectively, with the use of the Infinium II platform. Exclusion criteria included nonsteroidal anti-inflammatory drug use, subject relatedness, and failing genotyping quality control (described in Methods and in the online-only Data Supplement). Table 1 summarizes the demographics of the 116 AA and 125 EA subjects who were analyzed.

The PFA-100ColA phenotype was tested for association with each genotype under an additive model. With the use of log-transformed values of the PFA-100ColA phenotype, analysis was performed with the use of linear regression, controlling for sex, age, CD41, and VWF activity in AAs and for sex and VWF activity in EAs. For AAs, age ($P=\approx10^{-2}$), sex ($P=\approx10^{-2}$), CD41 ($P=\approx10^{-2}$), and VWF activity ($P=\approx10^{-9}$) were significant and were retained in the model. For EAs, only sex ($P=\approx10^{-2}$) and VWF activity ($P=\approx10^{-9}$) were significant, and therefore only these covariates were included in the analysis. Principal components analysis components were not included in the analysis because $\lambda=1.0$, indicating no inflation in the test statistic due to population substructure/admixture. Figure 1A shows Manhattan plots for genotype associations with PFA-100ColA closure times, with a prominent "peak" observed in chromosome 10 for AAs. Table 2 lists the SNPs with the 10 lowest *P* values for AAs. Notably, 4 of these SNPs were clustered within ≈21.8 kb of 4 exons within the *SVIL* gene, which encodes differentially spliced isoforms of supervillin and archvillin. The most significant SNP, rs7070678 ($P=3.6\times10^{-8}$), which is a synonymous SNP in exon 14 of the *SVIL* gene, is in linkage disequilibrium with other SNPs within the *SVIL* gene but not with SNPs in neighboring genes (Figure 1B). A second SNP, rs10826650, within the *SVIL* gene (intron 13) also reached GWAS significance ($P<5\times10^{-8}$). Two additional SNPs (rs7910521, rs10826649) within introns 16 and 17 of the *SVIL* gene had *P* values in the order of 10^{-6}. There was no evidence for deviation from the additive model (analyses not shown). The closest other well-annotated genes in this region, *LYZL1* (lysozyme-like 1) and *KIAA1462*, were >214 kb from the *SVIL* SNPs listed in Table 2, and there was no evidence of an association with either of these genes. The clustering of

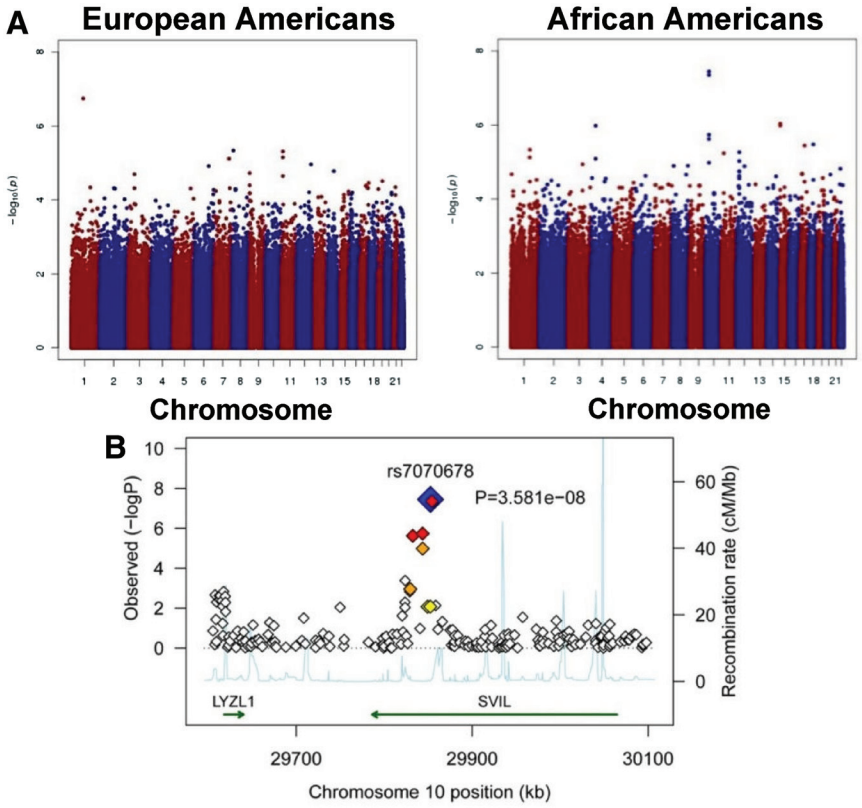

Figure 1. Genome-wide association studies with platelet function analyzer–100 closure times in a collagen- and ADP-impregnated cartridge (PFA-100ColA). **A**, Manhattan plots for PFA-100ColA. The Manhattan plot is shown for all autosomes for European and African Americans. The *y* axis shows the −log 10 of the *P* values for the chromosomes numbered on the *x* axis. For the African Americans, the 2 most significant single-nucleotide polymorphisms (SNPs) are rs7070678 and rs10826650 in supervillin (*SVIL*); both meet genome-wide significance with *P* values of 3.6×10^{-8} and 4.4×10^{-8}, respectively. None of the SNPs within *SVIL* meet genome-wide significance for European Americans. **B**, *SVIL* region association plot on chromosome 10 for African Americans. The most significant SNP rs7070678 is displayed, and the amount of linkage disequilibrium between this SNP and nearby SNPs is shown as a heat plot with SNPs in strongest linkage disequilibrium with rs7070678 in darker colors. The significance level is displayed on the *y* axis as −log 10 of the *P* values. The rate of recombination within the region is shown as the light blue tracing. The regions containing the *SVIL* and *LYZL1* genes are indicated by green arrows.

Table 2. Most Significant *P* Values Detected in Genotype Data From African Americans

SNP ID	Chromosome:Position	Genes	β	SE	MAF	Type	*P*
rs7070678	10:29812602	*SVIL*	−0.006807	0.001235	0.327	Exon 14 synonymous	3.581×10^{-8}
rs10826650	10:29814284	*SVIL*	−0.006856	0.001253	0.340	Intron 13	4.426×10^{-8}
rs11858159	15:24824188	*PWRN1*	0.005737	0.001169	0.366	Intron 7	9.1879×10^{-7}
rs3901472	15:24814582	*PWRN1*	0.005773	0.001182	0.379	Intron 3	1.034×10^{-6}
rs7656730	4:40159617	*N4BP2*	0.025637	0.005250	0.675	3′ UTR	1.044×10^{-6}
rs7910521	10:29803661	*SVIL*	−0.006104	0.001279	0.326	Intron 16	1.830×10^{-6}
rs10826649	10:29792539	*SVIL*	−0.006142	0.001301	0.297	Intron 17	2.382×10^{-6}
rs17783459	18:42329076	*SETBP1*	0.032067	0.006898	0.042	Intron 3	3.343×10^{-6}
rs9890514	17:46738883	None	0.012391	0.002675	0.196	. . .	3.604×10^{-6}
rs1507740	1:163075021	None	0.013215	0.002885	0.157	. . .	4.640×10^{-6}

SNP indicates single-nucleotide polymorphism; ID, identification; and MAF, minor allele frequency.

SNPs with *P* values of $\approx10^{-8}$ suggested that genetic variants in *SVIL* (and not another gene) are associated with PFA-100ColA closure times in AAs. No associations with GWAS significance were detected with SNP marker loci within the *SVIL* gene within EAs, although 3 additional *SVIL* SNPs had weak associations in EAs ($P<10^{-2}$).

Supervillin mRNA and Protein Are Present in Human and Mouse Platelets

Figure 2 illustrates the *SVIL* exon structure, the 2 major known mRNAs, and the location of rs7070678, the SNP with the strongest association with PFA-100ColA closure times. *SVIL* encodes supervillin, which has a broad cell distribution, and 2 archvillin isoforms, which are enriched in muscle.[22–24] Supervillin forms a high-affinity link between the actin cytoskeleton and the plasma membrane[22] but has not been described in platelets. Because integrin function and rearrangements of the actin cytoskeleton are a crucial aspect of the platelet adhesive process,[25] we therefore sought to characterize supervillin in platelets.

Platelet RNA expression analysis was performed with LDP RNA samples from 29 healthy subjects. Probes from the 5′-most and 3′-most exons (Figure 2A) were readily detectable. However, an RNA signal was not detected for probe ILMN_1659306, which is specific for archvillin and SmAV. Platelet *SVIL* transcripts are expressed at moderately high levels and are higher than platelet/endothelial cell adhesion molecule-1 but lower than GPIbα mRNAs (Figure I in the online-only Data Supplement). Supervillin transcripts were easily detected in LDP RNA by reverse transcription PCR (Figure 2B), validating the microarray data. Figure 2C shows *SVIL* mRNA levels for LDP, PRP, and $CD45^+$ leukocytes (white blood cells) as box plots.

Western immunoblotting identified supervillin at $\approx$205 kDa in PRP (Figure 3A). This polypeptide migrated slightly

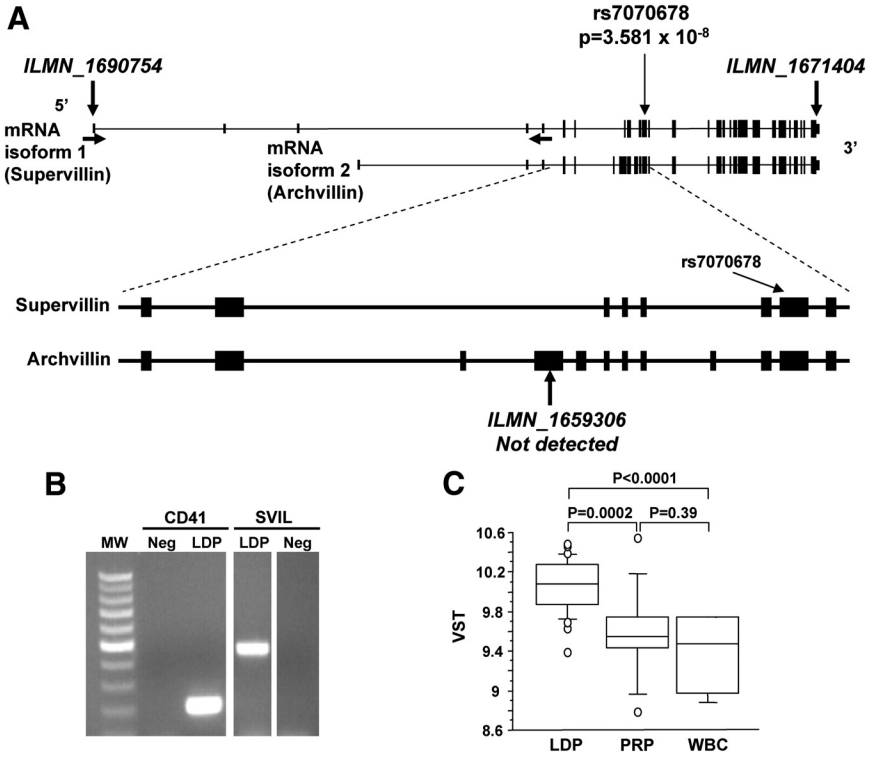

Figure 2. Supervillin (*SVIL*) genomic region and transcript expression. **A**, Exon structure of *SVIL* at chromosome 10:29786283–29963907 with exons of the 2 major transcripts (NM_003174, NM_021738) shown as lines and boxes. The location of the first of the 5 single-nucleotide polymorphisms (rs7070678) from Table 2 is shown by thin vertical arrows. The locations of the microarray probes are indicated by the thick vertical arrows and ILMN identification numbers. Horizontal arrows indicate the positions of the sense and antisense polymerase chain reaction primers. **B**, Ethidium-stained agarose gel showing reverse transcription polymerase chain reaction products of leukocyte-depleted platelet (LDP) RNA and primers specific for ITGA2 (CD41, integrin αIIb) and SVIL. Neg indicates no template control. **C**, Box plot showing the levels of *SVIL* mRNA (ILMN_1671404) in LDP (n=29), platelet-rich plasma (PRP) (n=11), and $CD45^+$ leukocytes (WBC; n=5). Box represents interquartile range, and whiskers represent 5% to 95% range. Variance stabilized transformation (VST) indicates natural log–transformed *SVIL* mRNA level.

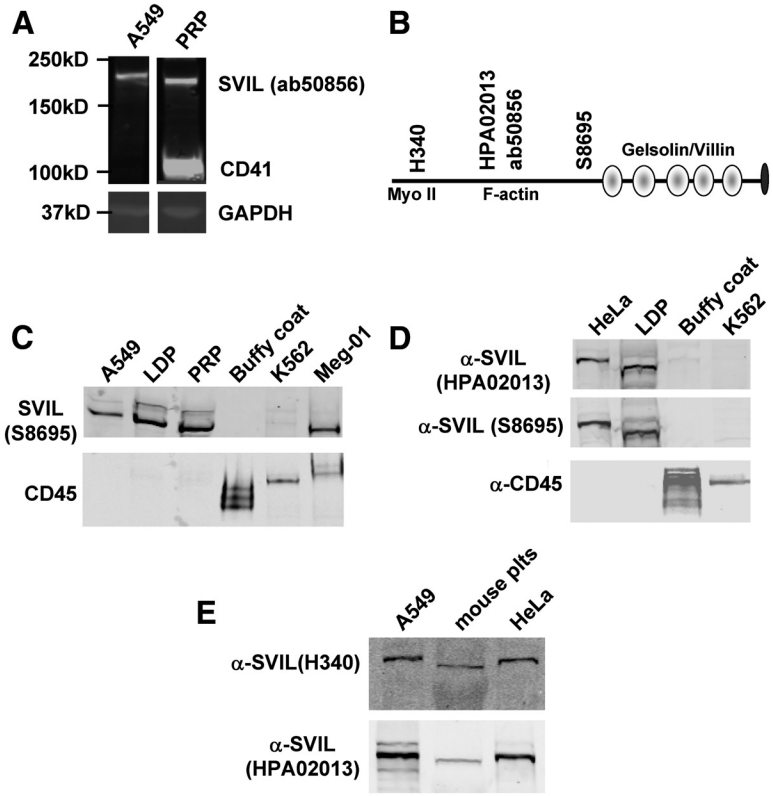

Figure 3. Platelet supervillin expression. **A**, Immunoblot of platelet-rich plasma (PRP) and, as a positive control, the A549 human alveolar adenocarcinoma cell line. Blots were probed with anti-supervillin (*SVIL*) and anti-CD41 (platelet integrin αIIb) antisera and imaged on a LI-COR Odyssey. GAPDH was loading control. **B**, Supervillin protein schematic showing locations of the gelsolin/villin homology repeats, the binding sites for myosin II heavy chain (Myo II) and F-actin, and the epitopes for the 4 antibodies used (H340, Ab50856, HPA02013, and S8695). **C**, Immunoblot probed with anti-*SVIL* antibody S8695 and anti-CD45. The erythroleukemia K562 and megakaryocytic Meg-01 cell lines are included. LDP indicates leukocyte-depleted platelets. **D**, Immunoblot with additional anti-*SVIL* antibodies. **E**, Immunoblot with H340 using lysates from mouse platelets (plts) and from A549 and HeLa adenocarcinoma cells as controls.

more quickly than that seen in control A549 cells, and therefore additional antisera were used to confirm platelet supervillin molecular weight and immunoreactivity. The locations of the epitopes recognized by these antibodies are shown in Figure 3B. In addition, because supervillin is present in leukocytes,[26,27] we also analyzed LDP and leukocyte-enriched buffy coat. With the use of 2 other antibodies, 205-kDa supervillin was again detected in plate-

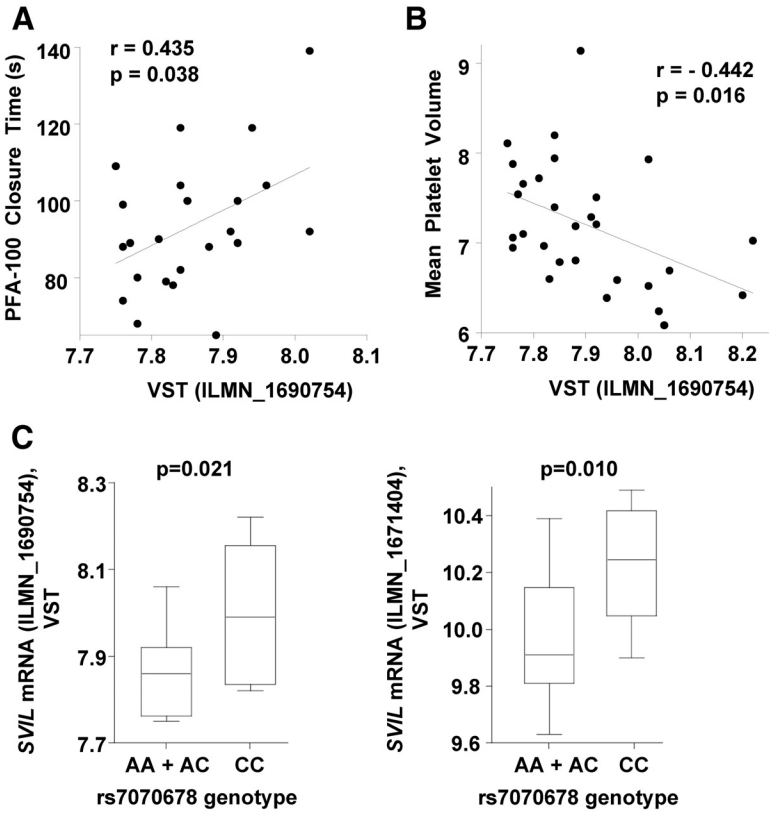

Figure 4. Correlation of supervillin (*SVIL*) mRNA levels with human platelet function. Natural log–transformed *SVIL* mRNA levels (VSTs) from gene expression profiling were correlated with platelet function analyzer–100 closure time (**A**), mean platelet volume (**B**), and *SVIL* expression levels (**C**) on the basis of rs7070678 genotype (n=29).

lets, as well as in the megakaryocyte cell line Meg-01, but was not detected in CD45-enriched leukocytes or K562 erythroleukemia cells (Figure 3C and 3D). Finally, supervillin was present in mouse platelets (Figure 3E). Sequencing of platelet *SVIL* mRNA reverse transcription PCR products revealed the presence of SVIL isoform 1, as in HeLa cells (not shown).

Supervillin mRNA Levels Correlate With Platelet Function Under Shear Stress, Platelet Size, and the rs7070678 Genotype

Because platelet protein was available from only a few of the 29 subjects used in the platelet RNA expression analysis, we used *SVIL* mRNA levels to test for a correlation with platelet function. Figure 4A shows that *SVIL* mRNA levels correlate with PFA-100ColA closure times ($P=0.038$). Because longer closure times indicate reduced platelet function, these data suggest that *SVIL* expression may inhibit human platelet thrombus formation under shear stress. A trend was observed for a similar relationship between *SVIL* mRNA expression and collagen-induced platelet aggregation ($P=0.07$) but not with ADP-induced platelet aggregation ($P=0.23$; data not shown). *SVIL* mRNA levels also correlated negatively with human mean platelet volume ($P=0.016$) (Figure 4B). Because both *SVIL* SNPs and *SVIL* transcripts were associated with PFA-100ColA closure times (Figures 1 and 4), we tested whether *SVIL* SNPs were associated with *SVIL* mRNA levels using a recessive model analysis. Figure 4C shows that *SVIL* expression differed significantly by rs7070678 genotype.

Platelets From *Svil*-Deficient Mice Form Thrombi Faster Under Shear Stress in Flow Chamber Studies

To further address the role of supervillin in platelet function, we generated mice from BayGenomics ES cells bearing an insertion in the supervillin (*Svil*) gene (Figure 5). In this mouse, a β-galactosidase/neomycin phosphotransferase (β-gal/neo) gene trap inserted into the large intron downstream of the 13th of the 34 coding exons (Figure 5A) disrupts expression of all characterized SVIL splice forms (not shown).[22–24] The location of the insertion was verified by Southern blotting, which showed that a *Bgl*II restriction site from the pGT0lxf vector reduced the 11.5-kb *Bgl*II fragment including coding exon 13 to 7.2 kb (not shown), and by PCR (Figure 5B) with primers specific for either the wild-type or mutated locus (Figure 5A, arrows). This insertion destabilizes the message or protein because supervillin is effectively absent from murine $Svil^{-/-}$ platelets (Figure 5C) and leukocytes (not shown).

To assess the role of supervillin in platelet thrombus formation under shear stress, we analyzed wild-type and $Svil^{-/-}$ platelets in microfluidic flow chamber assays on immobilized collagen.[21] No difference was observed under the "venous" flow rate of 400 s^{-1} (Figure 6A and 6B), but $Svil^{-/-}$ platelets showed greater deposition ($P<0.05$) under arterial flow rates of 1200 s^{-1} (Figure 6C and 6E). Both percent coverage of the collagen-coated area, indicating platelet adhesion to collagen, and sum intensity, indicating thrombus formation, were affected.

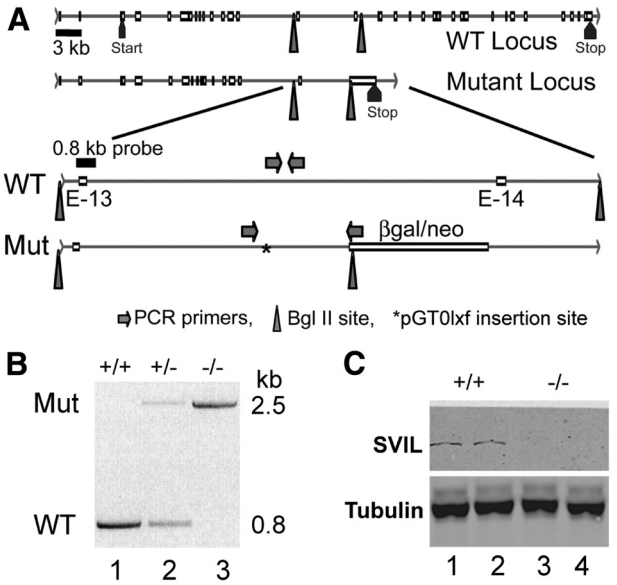

Figure 5. Disruption of the *Svil* gene and loss of supervillin (SVIL) expression in platelets. **A**, Diagram of wild-type (WT) and mutated (Mut) *Svil* loci (top 2 lines) with enlargements (bottom 2 lines) showing the insertion site (*) of the pGT0lxf vector sequence within the intron between coding exons 13 (E-13) and 14 (E-14). Also shown are the locations of polymerase chain reaction (PCR) primer sets diagnostic for the wild-type and mutant loci (arrows), the 0.8-kb probe used for Southern analyses, and the *Bgl*II sites (triangles) associated with endogenous genomic DNA and with the inserted β-galactosidase-neomycin (β-gal/neo) coding sequence. **B**, PCR of genomic DNA from mouse tails with the use of the primer sets shown in A. Primers specific for the insertion identify a ≈2.5-kb product (Mut, top), and primers specific for the wild-type allele generate a 0.8-kb product (WT, below). **C**, Immunoblot of mouse platelets with anti-SVIL antibody (H340) and anti-tubulin as a loading control.

Although there was no difference between wild-type and $Svil^{-/-}$ mice in platelet numbers, $Svil^{-/-}$ platelets appeared to be larger as measured by both forward scatter in flow cytometry and confocal microscopy (Table 3), consistent with the relationship in humans (Figure 4B). However, these larger platelets did not express correspondingly greater levels of major surface adhesion receptors integrin αIIb or GPIbα (Table 3).

Supervillin directly binds F-actin, myosin II heavy chain, filamin, and many other cytoskeletal proteins.[28–31] To better elucidate the cytoskeletal and structural differences between wild-type and $Svil^{-/-}$ platelets, we analyzed F-actin and myosin IIA organization using immunofluorescence confocal microscopy. Although these platelet cytoskeletons are similar in appearance when statically adhered to glass (Figure 7A and 7B versus 7C and 7D), $Svil^{-/-}$ platelets are more highly spread after activation under high shear flow across collagen (Figure 7E and 7F versus 7G and 7H). The intensity of F-actin staining increases after activation of both types of platelets, as expected given the associated polymerization of actin.[32,33]

Discussion

Interindividual variation in platelet reactivity contributes to common arterial thrombotic disorders in humans, but there is only a limited understanding of the responsible molecular

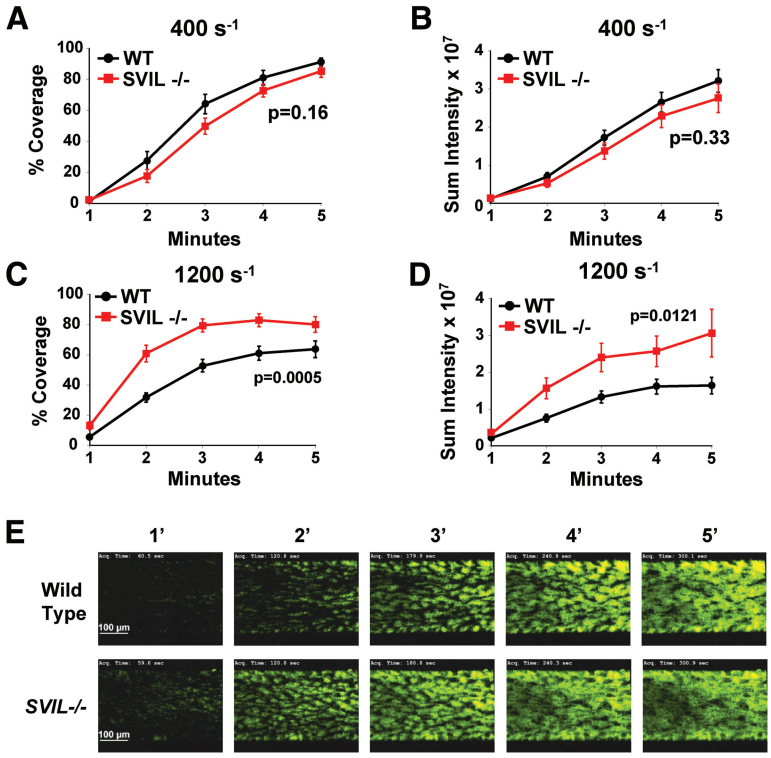

Figure 6. Enhanced adhesion and thrombus formation in platelets lacking supervillin (SVIL). Whole blood from wild-type (WT; black line) or *Svil*$^{-/-}$ (SVIL; red line) mice was perfused over immobilized collagen at venous (400 s^{-1}) or arterial (1200 s^{-1}) shear conditions. Platelets in whole blood were labeled with AlexaFluor488-labeled antibodies to platelet GPIX before perfusion. **A** and **C**, Surface area covered by platelets at indicated time points presented as percentage of coated collagen ±SEM (n=15 for each genotype). **B** and **D**, Sum of fluorescence intensity ±SEM measured at the indicated time points (n=15 for each genotype). Experiments shown in A to D were conducted on 4 different days for a total of 15 runs for each genotype. **E**, Representative images were taken at the designated times during perfusion at arterial (1200 s^{-1}) shear rates. Bars shown in 1-minute images apply to all time points.

mechanisms. We screened a cohort of healthy human subjects by genome-wide genotyping for associations with platelet function assessed under shear stress and identified a candidate gene in AAs that was validated using gene expression and platelet physiology approaches. Our major findings were that (1) genetic variants in *SVIL* were associated with closure times in the PFA-100ColA; (2) platelets contain supervillin and little or no archvillin or SmAV; and (3) low or absent platelet supervillin is associated with enhanced thrombus formation under high shear stress and increased platelet size in both humans and mice.

Growing evidence supports a role for supervillin as a regulator of cytoskeletal-membrane interactions. Among other functions, supervillin increases myosin II contractility, reduces integrin-mediated cell adhesion, and promotes rapid integrin recycling.[34–36] Our data support an inhibitory role for supervillin in regulating the rapid activation and spreading of platelets during thrombus formation on collagen under shear stress, an important determinant of platelet responsiveness in arterial thrombosis. These results are consistent with supervillin inhibition of spreading and integrin function in other cell types.[37,38]

Table 3. Platelet Parameters in Wild-Type and *Svil*$^{-/-}$ Mice

Parameter	Wild-Type	*Svil*$^{-/-}$	*P**
Platelet No./µL†	1 083 000	969 000	0.67
Mean forward scatter‡	19.4	23.2	<0.0001
Surface area, µm^2§	4.7±0.1 (n=159)	5.3±0.1 (n=241)	0.0045
Integrin αIIb, MFI fold change‖	1.02		0.89
Glycoprotein Ibα, MFI fold change‖	1.30		0.40

MFI indicates mean fluorescence intensity ratio of mutant/wild-type platelets.

*Calculated by Student *t* test (unless otherwise indicated, n=6 for each genotype).

†By Hemavet 850FS (Drew Scientific).

‡By FACScan (Becton Dickinson).

§Mean±SEM by confocal microscopy of AlexaFluor568-phalloidin stained unactivated.

‖Flow cytometric comparisons between mice are shown as fold changes (WT/SVIL) to normalize for day-to-day variation in platelets, antibody fluorescence, binding, and acquisition.

Genome-Wide Association Study

The PFA-100 is dependent on shear stress and is relatively "high throughput." We appreciate that some[12–15] but not all[39,40] clinical cardiovascular outcome studies have observed associations with PFA-100 results. The lack of an association between PFA-100 results and clinical outcomes may be due to some nonphysiological conditions, such as anticoagulated blood or an absence of vessel wall. It would be ideal to replicate our findings with other assays in humans; however, neither light transmission aggregometry nor impedance aggregometry is performed under conditions considered to apply shear stress to platelets. Our strategy was to use the GWAS as a screen for identifying novel genes associated with shear-dependent platelet reactivity. However, our study differs from prior platelet genomics studies in that our validation approach employed mRNA expression and physiology experiments. Our statistical analysis was strengthened by the ability to adjust for confounders known to affect platelet adhesion (VWF activity) and aggregation (CD41 levels), which were also shown to be significant in the analysis. Two SNPs in *SVIL* met the threshold for genome-wide significance in AAs, and 2 additional *SVIL* SNPs were associated with PFA-100ColA closure times, with *P* values of ~10^{-6}.

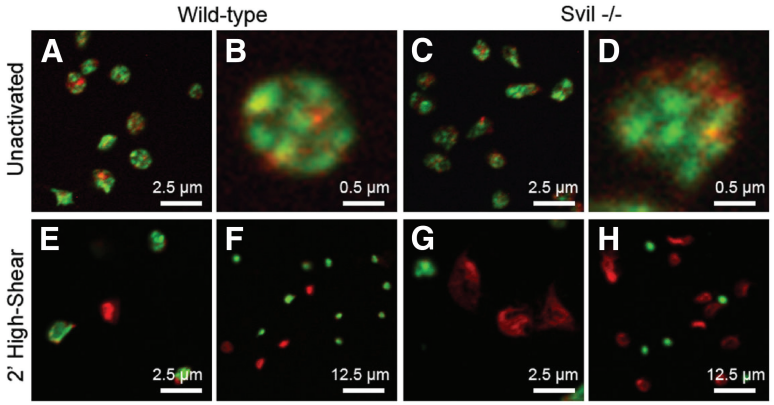

Figure 7. Immunofluorescence micrographs of wild-type (**A**, **B**, **E**, **F**) or supervillin (Svil)-deficient (**C**, **D**, **G**, **H**) platelets fixed statically onto glass (**A** through **D**) or after activation for 2 minutes on collagen under high-shear (1200 s^{-1}) flow (**E** through **H**). F-actin (red) was visualized with AlexaFluor568-phalloidin; myosin IIA (green) was stained with antibody. Bars=2.5, 0.5, and 12.5 μm, as indicated.

The reason for our inability to detect a similarly strong association in EAs is unclear but probably relates to the limited power of a small sample size by typical GWAS standards and the fact that minor allele frequencies were ≈20% lower in EAs. Thus, much larger sample sizes might be necessary to detect an association in EAs. Indeed, supplemental data in the genomic analysis by Johnson et al[9] show modest associations ($\approx 10^{-5}$) between SNPs in *SVIL* and in vitro platelet aggregation in both AAs and EAs.

None of the 5 *SVIL* SNPs associated with PFA-100ColA closure times were predicted to affect the protein coding sequence, and therefore we had no reason to suspect that any of these SNPs directly altered supervillin function. This is not unusual because GWAS uses indirect association mapping with tag SNPs that are in linkage disequilibrium with causal variants.[8] However, despite a small sample size composed of both EAs and AAs in our RNA expression study, expression quantitative trait loci analysis supported an association between these SNPs and *SVIL* mRNA levels (Figure 4C). Thus, at least 1 genetic mechanism by which *SVIL* variants may regulate platelet reactivity is by altering *SVIL* expression, perhaps via effects on transcription or mRNA stability.

SVIL Expression in Platelets

SVIL mRNA was detected in platelets with the use of a 3′UTR probe that recognizes all *SVIL* transcripts and a 5′UTR probe specific for nonmuscle supervillin (Figure 2). Archvillin mRNAs were not detected in our microarray expression study, consistent with prior data indicating that archvillin and SmAV are mainly expressed in muscle.[23] In addition to the RNA data, immunoblotting with multiple antibodies demonstrated the presence of abundant supervillin protein in platelets. Using microarray analysis, Watkins et al[27] reported that *SVIL* mRNA is expressed in multiple hematopoietic cell types including $CD4^+$ and $CD8^+$ T cells, $CD14^+$ monocytes, $CD19^+$ B cells, $CD56^+$ natural killer cells, and $CD66b^+$ granulocytes. We also observed *SVIL* mRNA in $CD45^+$ cells (Figure 2C); found supervillin protein in murine thymocytes, splenocytes, macrophages, and neutrophils by immunoblotting; and recovered Coomassie blue–staining amounts of supervillin from bovine neutrophils.[26] Although high levels of proteases may have caused degradation in human leukocyte lysates (Figure 3), supervillin is clearly expressed at moderately high levels in platelets (Figures 2C and 3 and Figure I in the online-only Data Supplement). In addition, because PRP has some contamination with white blood cells, our gene expression data suggest that *SVIL* mRNA is expressed at higher levels in platelets than in white blood cells (Figure 2C). The basis for the reproducibly faster migration of platelet supervillin, compared with HeLa and A549 cell supervillin (Figure 3), is unclear because the predicted mRNAs are identical. A likely explanation is a cell type–specific difference in posttranslational modifications.

Supervillin and Platelet Size

Supervillin expression levels are inversely correlated with platelet size in both humans and mice. Although $Svil^{-/-}$ platelets are larger, they do not have greater surface expression of several critical adhesive glycoproteins (GPIbα or integrin α_{IIb}), suggesting a possible membrane cytoskeletal defect rather than premature release of larger platelets from megakaryocytes. There are several possible mechanisms by which supervillin might regulate platelet size. Filamin anchors the GPIb-IX-V complex to the platelet cytoskeleton and binds to supervillin[31,41]; an altered filamin-GPIbα interaction could produce larger platelets, such as those characteristic of inherited mutations in the gene encoding GPIbα.[42,43] Other possible mechanisms include altered myosin II function similar to the *MYH9*-associated macrothrombocytopenias[44] or a simple disruption of the actin cytoskeleton that maintains normal platelet size, as in the Wiskott-Aldrich syndrome.[45] The increased platelet size may enhance thrombus formation because high platelet volumes have been associated with MI.[16]

Supervillin and Platelet Function

Supervillin effects on platelet function are most prominent under shear stress. Healthy human subjects expressing higher levels of *SVIL* mRNA exhibit slower platelet thrombus formation in the PFA-100ColA. Platelets in blood from supervillin-deficient mice form thrombi faster under high shear rates in flow chamber studies than is observed for platelets with wild-type *Svil* (Figure 6). Although the effect of supervillin on platelet adhesion is not dramatic, it is difficult to demonstrate gain-of-function effects, and the increased adhesion observed in *Svil*-null platelets is well in agreement with other mouse mutants of signaling molecules that limit

platelet activation.[37,38] The mice used in these studies have *Svil* defects in all tissues, and we cannot exclude an indirect effect on platelets. However, the *SVIL* association studies used here and by Johnson et al[9] utilized an in vitro platelet assay consisting only of platelets and plasma (ie, aggregometry). Taken together, our results strongly suggest an inhibitory role for supervillin in platelet function.

An inhibitory effect during spreading is consistent with the larger surface profiles observed for supervillin-deficient platelets on collagen after 2 minutes of flow under high shear stress (Figure 7). The direction of this effect is consistent with a number of mechanisms. First, the larger initial volumes of unactivated $Svil^{-/-}$ platelets (Table 3) may contribute to their larger surface areas after activation. Second, the loss of supervillin could increase the rate of cell spreading by increasing integrin adhesion to the substrate or by decreasing myosin II–mediated slowing of the cell spreading rate, as observed in other cell types.[34,35] Finally, decreased integrity of the membrane-cytoskeleton connection could increase the apparent cross-sectional surface area if $Svil^{-/-}$ platelets are more sensitive to cortical disruption by high shear forces. Further studies are needed to determine which supervillin interactions regulate the early phases of platelet activation and adhesion under high-shear forces.

There are potential clinical implications to our findings. If confirmed in additional studies, these data suggest that *SVIL* variants may contribute to predisposition to cardiovascular disease in AAs. Targeting supervillin in a manner that would enhance its activity might have antithrombotic benefit for arterial vascular diseases such as MI and stroke, a benefit that may be more pronounced in AAs. Conversely, drugs that interrupt supervillin function in platelets could have untoward effects of promoting thrombosis. Finally, SNPs in strong linkage disequilibrium with the causative *SVIL* variant may be useful as a biomarker for risk of thrombosis or hemorrhage.

Summary

Combined genome-wide technologies in a cohort with well-characterized platelet function have led to the identification of a novel protein in platelet biology. We have identified a candidate gene meeting GWAS significance, and both human and mouse studies support an inhibitory effect of supervillin in platelet thrombus formation under shear stress. Although the causative variant in *SVIL* has not been identified, these data indicate that genetic variations in *SVIL* expression contribute to variations in human platelet reactivity and support a role for supervillin in arterial thrombosis. Further analysis of *SVIL* variants may lead to a better understanding of the genetic basis for susceptibility to arterial thrombosis.

Acknowledgments

The authors wish to thank Jing-Fei Dong for supervising platelet phenotype acquisition and helpful discussions; Angela Bergeron, Carol Sun, Lucia Stefanini, and Lin Ma for technical support; Debra Newman for generously providing platelet/endothelial cell adhesion molecule-1; and Paul Furcinitti (University of Massachusetts Medical School Biomedical Imaging Facility) for assistance in acquiring confocal images.

Sources of Funding

This work was supported by National Institutes of Health grants HL88458 (Dr Bray), GM-033048 (Dr Luna), and HL094594 (Dr Bergmeier) and by Muscular Dystrophy Association grant 3160 (Dr Luna).

Disclosures

None.

References

1. Ruggeri ZM. Platelets in atherothrombosis. *Nat Med*. 2002;8:1227–1234.
2. Fredrickson BJ, Dong JF, McIntire LV, López JA. Shear-dependent rolling on von Willebrand factor of mammalian cells expressing the platelet glycoprotein Ib-IX-V complex. *Blood*. 1998;92:3684–3693.
3. Nesbitt WS, Westein E, Tovar-Lopez FJ, Tolouei E, Mitchell A, Fu J, Carberry J, Fouras A, Jackson SP. A shear gradient-dependent platelet aggregation mechanism drives thrombus formation. *Nat Med*. 2009;15:665–673.
4. Bray PF, Mathias RA, Faraday N, Yanek LR, Fallin MD, Herrera-Galeano JE, Wilson AF, Becker LC, Becker DM. Heritability of platelet function in families with premature coronary artery disease. *J Thromb Haemost*. 2007;5:1617–1623.
5. Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. *N Engl J Med*. 1994;330:1041–1046.
6. Ouwehand WH. The discovery of genes implicated in myocardial infarction. *J Thromb Haemost*. 2009;7:305–307.
7. Li W, Wang M, Irigoyen P, Gregersen PK. Inferring causal relationships among intermediate phenotypes and biomarkers: a case study of rheumatoid arthritis. *Bioinformatics*. 2006;22:1503–1507.
8. Manolio TA, Brooks LD, Collins FS. A hapmap harvest of insights into the genetics of common disease. *J Clin Invest*. 2008;118:1590–1605.
9. Johnson AD, Yanek LR, Chen MH, Faraday N, Larson MG, Tofler G, Lin SJ, Kraja AT, Province MA, Yang Q, Becker DM, O'Donnell CJ, Becker LC. Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. *Nat Gen*. 2010;42:608–613.
10. Soranzo N, Rendon A, Gieger C, Jones CI, Watkins NA, Menzel S, Doring A, Stephens J, Prokisch H, Erber W, Potter SC, Bray SL, Burns P, Jolley J, Falchi M, Kuhnel B, Erdmann J, Schunkert H, Samani NJ, Illig T, Garner SF, Rankin A, Meisinger C, Bradley JR, Thein SL, Goodall AH, Spector TD, Deloukas P, Ouwehand WH. A novel variant on chromosome 7q22.3 associated with mean platelet volume, counts, and function. *Blood*. 2009;113:3831–3837.
11. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an in vitro platelet function analyzer: PFA-100. *Semin Thromb Hemost*. 1995;21:106–112.
12. Ziegler S, Maca T, Alt E, Speiser W, Schneider B, Minar E. Monitoring of antiplatelet therapy with the PFA-100(R) in peripheral angioplasty patients. *Platelets*. 2002;13:493–497.
13. Frossard M, Fuchs I, Leitner JM, Hsieh K, Vlcek M, Losert H, Domanovits H, Schreiber W, Laggner AN, Jilma B. Platelet function predicts myocardial damage in patients with acute myocardial infarction. *Circulation*. 2004;110:1392–1397.
14. Gianetti J, Parri MS, Sbrana S, Paoli F, Maffei S, Paradossi U, Berti S, Clerico A, Biagini A. Platelet activation predicts recurrent ischemic events after percutaneous coronary angioplasty: a 6 months prospective study. *Thromb Res*. 2006;118:487–493.
15. Fuchs I, Frossard M, Spiel A, Riedmuller E, Laggner AN, Jilma B. Platelet function in patients with acute coronary syndrome (ACS) predicts recurrent ACS. *J Thromb Haemost*. 2006;4:2547–2552.
16. Bray PF. Platelet hyperreactivity: predictive and intrinsic properties. *Hematol Oncol Clin North Am*. 2007;21:633–645.
17. Favaloro EJ. Clinical application of the PFA-100. *Curr Opin Hematol*. 2002;9:407–415.
18. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904–909.
19. Nagalla S, Shaw C, Kong X, Kondkar AA, Edelstein LC, Ma L, Chen J, McKnight GS, Lopez JA, Yang L, Jin Y, Bray MS, Leal SM, Dong JF, Bray PF. Platelet microRNA-mRNA coexpression profiles correlate with platelet reactivity. *Blood*. 2011;117:5189–5197.
20. Kondkar AA, Bray MS, Leal SM, Nagalla S, Liu DJ, Jin Y, Dong JF, Ren Q, Whiteheart SW, Shaw C, Bray PF. VAMP8/endobrevin is overex-

pressed in hyperreactive human platelets: suggested role for platelet microRNA. *J Thromb Haemost*. 2010;8:369–378.
21. Stolla M, Stefanini L, Roden RC, Chavez M, Hirsch J, Greene T, Ouellette TD, Maloney SF, Diamond SL, Poncz M, Woulfe DS, Bergmeier W. The kinetics of alphaIIbbeta3 activation determines the size and stability of thrombi in mice: implications for antiplatelet therapy. *Blood*. 2011;117:1005–1013.
22. Pestonjamasp KN, Pope RK, Wulfkuhle JD, Luna EJ. Supervillin (p205): a novel membrane-associated, F-actin-binding protein in the villin/gelsolin superfamily. *J Cell Biol*. 1997;139:1255–1269.
23. Oh SW, Pope RK, Smith KP, Crowley JL, Nebl T, Lawrence JB, Luna EJ. Archvillin, a muscle-specific isoform of supervillin, is an early expressed component of the costameric membrane skeleton. *J Cell Sci*. 2003;116:2261–2275.
24. Gangopadhyay SS, Takizawa N, Gallant C, Barber AL, Je HD, Smith TC, Luna EJ, Morgan KG. Smooth muscle archvillin: a novel regulator of signaling and contractility in vascular smooth muscle. *J Cell Sci*. 2004;117:5043–5057.
25. Nuyttens BP, Thijs T, Deckmyn H, Broos K. Platelet adhesion to collagen. *Thromb Res*. 2011;127:S26–S29.
26. Nebl T, Pestonjamasp KN, Leszyk JD, Crowley JL, Oh SW, Luna EJ. Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes. *J Biol Chem*. 2002;277:43399–43409.
27. Watkins NA, Gusnanto A, de Bono B, De S, Miranda-Saavedra D, Hardie DL, Angenent WG, Attwood AP, Ellis PD, Erber W, Foad NS, Garner SF, Isacke CM, Jolley J, Koch K, Macaulay IC, Morley SL, Rendon A, Rice KM, Taylor N, Thijssen-Timmer DC, Tijssen MR, van der Schoot CE, Wernisch L, Winzer T, Dudbridge F, Buckley CD, Langford CF, Teichmann S, Gottgens B, Ouwehand WH. A HaemAtlas: characterizing gene expression in differentiated human blood cells. *Blood*. 2009;113:e1–e9.
28. Chen Y, Takizawa N, Crowley JL, Oh SW, Gatto CL, Kambara T, Sato O, Li XD, Ikebe M, Luna EJ. F-actin and myosin II binding domains in supervillin. *J Biol Chem*. 2003;278:46094–46106.
29. Wulfkuhle JD, Donina IE, Stark NH, Pope RK, Pestonjamasp KN, Niswonger ML, Luna EJ. Domain analysis of supervillin, an F-actin bundling plasma membrane protein with functional nuclear localization signals. *J Cell Sci*. 1999;112(pt 13):2125–2136.
30. Crowley JL, Smith TC, Fang Z, Takizawa N, Luna EJ. Supervillin reorganizes the actin cytoskeleton and increases invadopodial efficiency. *Mol Biol Cell*. 2009;20:948–962.
31. Smith TC, Fang Z, Luna EJ. Novel interactors and a role for supervillin in early cytokinesis. *Cytoskeleton (Hoboken)*. 2010;67:346–364.
32. Jennings LK, Fox JE, Edwards HH, Phillips DR. Changes in the cytoskeletal structure of human platelets following thrombin activation. *J Biol Chem*. 1981;256:6927–6932.
33. Loftus JC, Choate J, Albrecht RM. Platelet activation and cytoskeletal reorganization: high voltage electron microscopic examination of intact and triton-extracted whole mounts. *J Cell Biol*. 1984;98:2019–2025.
34. Takizawa N, Ikebe R, Ikebe M, Luna EJ. Supervillin slows cell spreading by facilitating myosin II activation at the cell periphery. *J Cell Sci*. 2007;120:3792–3803.
35. Takizawa N, Smith TC, Nebl T, Crowley JL, Palmieri SJ, Lifshitz LM, Ehrhardt AG, Hoffman LM, Beckerle MC, Luna EJ. Supervillin modulation of focal adhesions involving TRIP6/ZRP-1. *J Cell Biol*. 2006;174:447–458.
36. Fang Z, Takizawa N, Wilson KA, Smith TC, Delprato A, Davidson MW, Lambright DG, Luna EJ. The membrane-associated protein, supervillin, accelerates F-actin-dependent rapid integrin recycling and cell motility. *Traffic*. 2010;11:782–799.
37. Falati S, Patil S, Gross PL, Stapleton M, Merrill-Skoloff G, Barrett NE, Pixton KL, Weiler H, Cooley B, Newman DK, Newman PJ, Furie BC, Furie B, Gibbins JM. Platelet PECAM-1 inhibits thrombus formation in vivo. *Blood*. 2006;107:535–541.
38. Signarvic RS, Cierniewska A, Stalker TJ, Fong KP, Chatterjee MS, Hess PR, Ma P, Diamond SL, Neubig RR, Brass LF. RGS/Gi2alpha interactions modulate platelet accumulation and thrombus formation at sites of vascular injury. *Blood*. 2010;116:6092–6100.
39. Modica A, Karlsson F, Mooe T. The impact of platelet function or C-reactive protein, on cardiovascular events after an acute myocardial infarction. *Thromb J*. 2009;7:12.
40. Breet NJ, van Werkum JW, Bouman HJ, Kelder JC, Ruven HJ, Bal ET, Deneer VH, Harmsze AM, van der Heyden JA, Rensing BJ, Suttorp MJ, Hackeng CM, ten Berg JM. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. *JAMA*. 2010;303:754–762.
41. Takafuta T, Wu G, Murphy GF, Shapiro SS. Human beta-filamin is a new protein that interacts with the cytoplasmic tail of glycoprotein Ibalpha. *J Biol Chem*. 1998;273:17531–17538.
42. Lopez JA, Andrews RK, Afshar-Kharghan V, Berndt MC. Bernard-Soulier syndrome. *Blood*. 1998;91:4397–4418.
43. Kunishima S, Kamiya T, Saito H. Genetic abnormalities of Bernard-Soulier syndrome. *Int J Hematol*. 2002;76:319–327.
44. Kunishima S, Saito H. Advances in the understanding of MYH9 disorders. *Curr Opin Hematol*. 2010;17:405–410.
45. Thrasher AJ. New insights into the biology of Wiskott-Aldrich syndrome (WAS). *Hematology Am Soc Hematol Educ Program*. 2009:132–138.

CLINICAL PERSPECTIVE

Platelets play a central role in ischemic arterial vascular disease, and antiplatelet therapies are mainstays of treatment. The findings in this study identify a novel platelet protein, supervillin, which functions to dampen the early formation of platelet thrombi under high shear stress. Although these results will not alter current management of vascular disease, there are potential clinical implications. Supervillin is an interaction hub for many proteins that regulate cell adhesion and contractility. Drug targeting of supervillin or one of its binding partners in a manner that would decrease platelet adhesion under high shear forces may have antithrombotic benefit for arterial vascular diseases such as myocardial infarction and stroke. This benefit could be especially pronounced in African Americans, who suffer disproportionately from cardiovascular disease. Conversely, drugs that knowingly or unknowingly block supervillin function in platelets could have untoward effects of promoting thrombosis. The shear dependence of the supervillin effect presents an opportunity to develop therapies that differentially affect arterial and venous thrombosis by inhibiting platelet thrombus formation under high shear settings (eg, acute coronary syndromes or percutaneous coronary intervention) without altering the normal hemostatic function of platelets in low-shear veins or microcirculation. Finally, single-nucleotide polymorphisms strongly linked to the causative supervillin variant may be useful as a biomarker for risk of thrombosis or hemorrhage.

Go to http://cme.ahajournals.org to take the CME quiz for this article.