Abstract—Understanding genetic contributions to platelet function could have profound clinical ramifications for personalizing platelet-directed pharmacotherapy, by providing insight into the risks and possible benefits associated with specific genotypes. This article represents an integrated summary of presentations related to genetic regulation of platelet receptor expression and function given at the Fifth Annual Platelet Colloquium in January 2010. It is supplemented with additional highlights from the literature covering (1) approaches to determining and evidence for the associations of genetic variants with platelet hypo- and hyperresponsive phenotypes, (2) the ramifications of these polymorphisms with regard to clinical responses to antiplatelet therapies, and (3) the role of platelet function/genetic testing in guiding antiplatelet therapy. (Arterioscler Thromb Vasc Biol. 2010;30:2372-2384.)

Key Words: gene expression • hemostasis • platelets • receptors • thrombosis

Platelet aggregation is a key component for development of acute thrombosis in coronary, cerebral, and peripheral arterial diseases. Endogenous and environmental factors—age, cholesterol levels, hypertension, diabetes mellitus, and cigarette smoking—explain only part of the variation in platelet function observed in persons with these conditions. Although inherited and genetic factors have known links to bleeding disorders and prothrombotic phenotypes, the evidence for genetic influences that enhance platelet function is much weaker. Understanding the genetic contributions to platelet function could have profound clinical ramifications for personalizing platelet-directed pharmacotherapy, by providing insight into the risks and possible benefits associated with specific genotypes.

This review, based on information presented at the fifth annual Platelet Colloquium held in Washington, DC, in January 2010, focuses on the genetic regulation of and variations in platelet receptor expression, function, and responses to antiplatelet therapies and how emerging knowledge in these areas might be applied clinically.

Evidence for Genetic Regulation of Platelet Function

Several well-characterized inherited disorders result from molecular defects that disrupt platelet function and therefore lead to bleeding phenotypes. Studies of platelet-related bleeding disorders, such as Glanzmann thrombasthenia, caused by mutations in integrins αIb (glycoprotein [GP] IIb) or β3 (GP IIIa), and Bernard Soulier syndrome, caused by mutations in GP Ib, have provided important insight into platelet function.

Focus has recently shifted to understanding genetic variants that might enhance platelet function. Although definitions for platelet responsiveness tend to differ among studies, it is now widely accepted that platelet aggregation ex vivo in response to agonist stimulation varies considerably among healthy individuals. In an analysis of 359 healthy people, Yee et al1 noted that a minority consistently showed hyperresponsiveness (>65% maximal platelet aggregation) after stimulation with ADP, collagen, epinephrine, collagen-related peptide, or ristocetin. Female sex and higher fibrinogen levels...
were significantly associated with hyperresponsiveness,\textsuperscript{1} and hyperreactivity to 1 agonist tended to persist with others in the assays studied. Several epidemiological and twin studies suggest that the extent of platelet aggregability may be heritable.\textsuperscript{2–9} Analysis of 2413 subjects without known atherosclerotic disease in the Framingham Heart Study showed significant correlation in platelet aggregation among siblings in response to epinephrine, ADP, and collagen lag time.\textsuperscript{10} Similarly, a study of 1008 Americans who had $\geq1$ family member with premature coronary artery disease (CAD), which included a family history of early myocardial infarction (MI) and sudden cardiac death, showed evidence for moderate to strong heritability in epinephrine- and ADP-induced aggregation responses ($h^2$ of 0.36 to 0.42 in white subjects and $>0.71$ in black subjects).\textsuperscript{11} In this latter study, the contribution from established cardiac risk factors to any given platelet phenotype was smaller than that from platelet-specific factors. Although by no means conclusive, these studies suggest an inherited component to platelet responses that may predispose individuals to acute arterial thrombosis.

The next section reviews approaches to determining molecular variants associated with enhanced platelet responses, including candidate gene-association studies, genome-wide association studies (GWAS), and assessment of gene expression by messenger RNA (mRNA) profiling. It will soon be possible to perform individual genome (DNA) sequencing or transcriptome (RNA) analysis. For all of the approaches discussed below, the importance of careful phenotyping for interpretation of genetic associations cannot be overemphasized.

**Selected Platelet Polymorphisms and Platelet Function**

A brief summary of some of the more prominent candidate genes is presented below. The section provides examples of some of the observations and controversies in the field and is not meant to be an exhaustive cataloging of all available data. For additional information on candidate genes associated with differences in platelet phenotypes, readers are referred to a recent comprehensive review on this topic.\textsuperscript{12}

**Glycoprotein Ia/Ii ({$\alpha^2\beta^1$})**

The rate of platelet attachment to type I collagen under conditions of high shear relates directly to the density of GP Ia/Ii ({$\alpha^2\beta^1$}) receptor; if the density is high, there may be a propensity for thrombosis, and if it is low, the risk of bleeding may be increased.\textsuperscript{13} Several polymorphisms exist in the coding region for this gene. Two silent polymorphisms are in complete linkage disequilibrium—807C$>$T and 873G$>$A—and 2 others show linkage disequilibrium—837C$>$T and 1648A$>$G (human platelet antigen [HPA]–Br$^b$).\textsuperscript{14} Most recently, a new polymorphism has been identified in the 5’ regulatory region of the {$\alpha^2$} gene (52T$>$C).\textsuperscript{15} The 807T allele is associated with increased density of the GP Ia/Ii receptor, and the presence of the 807C allele is associated with reduced receptor density.\textsuperscript{14,15} Figure 1 illustrates the relationship between specific variants of this gene and receptor density as shown on real-time epifluorescence video microscopy.\textsuperscript{13}

Table 1 summarizes the clinical studies examining the association between the 807T$>$C variant and thrombotic disorders.\textsuperscript{16–41} For CAD, other arterial thrombosis, major adverse cardiac events within 30 days after stenting, and venous thrombosis, studies have generally not shown a significant link with the 807T allele. In the most recent meta-analyses, the 807T allele was not shown to be a significant risk factor for CAD,\textsuperscript{42,43} although evidence is split for an association with the risk for ischemic stroke.\textsuperscript{27–33} Polymorphisms such as 807T, which are located in the coding region of the {$\alpha^2$} gene, also might interact with variants in the regulatory region, such as $-52C$,$-T$ and $-92C$,$-G$, to alter changes in receptor density.\textsuperscript{15} Finally, given the wide range in frequency of variants among populations,\textsuperscript{40,44} it is critical to select the appropriate controls when evaluating genetic contributions to vascular disease risk. This latter phenomenon and publication bias may contribute to some of the conflicting results in the literature.

**Glycoprotein Iba**

The major function of the GP Ib-IX-V receptor complex relates to adhesion of platelets to immobilized von Willebrand factor in areas of high shear stress, resulting in platelet activation. The complex also binds thrombin and P-selectin and mediates platelet–leukocyte interactions,\textsuperscript{45} and the subunits are encoded by distinct genes. Four of the known polymorphisms of the gene coding GP Iba are categorized by the variable number of tandem repeats (VNTR-A to VNTR-D) of a 39-bp sequence.\textsuperscript{46} Another (VNTR-E) appears to be a deletion mutation, with no bp sequence repeated,\textsuperscript{47} and the HPA-2$^{ab}$ (Ko) polymorphism, consisting of a C$>$T transition at nucleotide 1018, results in a single amino-acid substitution at residue 145 (Thr$^b$>Met$^b$).\textsuperscript{48} This polymorphism shows strong linkage disequilibrium with the VNTR polymorphisms.\textsuperscript{48} Platelet plug formation under high shear stress may be influenced by the VNTR-C$D$ versus -CC genotype.\textsuperscript{49} The HPA-2 (Ko) polymorphism has been associated with higher affinity for von Willebrand factor ristocetin- or botrocetin-induced binding conditions, but this variant does not appear to affect $\alpha$-thrombin binding.\textsuperscript{49}

Several clinical studies have assessed the functional effects of these polymorphisms (Tables 2 and 3).\textsuperscript{25,30,32–35,50–71} Although these studies have shown conflicting results, the preponderance of the evidence indicates a lack of significant association of the VNTR and HPA-2 polymorphisms with MI, stroke, CAD, and venous thromboembolism. In a recent meta-analysis of 8 studies, the presence of the HPA-2$^b$ allele was associated with an adjusted OR of 1.43 (95% CI, 1.13 to 1.81) for ischemic stroke.\textsuperscript{72}

**Glycoprotein IIb/IIia**

The integrin $\alpha_{\text{IIb}}$\$\beta_3$ receptor binds fibrinogen, von Willebrand factor, fibronectin, and vitronectin. The primary polymorphism for this receptor is the substitution of proline for leucine at position 33 (T1565C; PlA1/PlA2).\textsuperscript{73} The presence of the PlA1 allele has been associated with increases in P-selectin, fibrinogen, and activated GP IIb/IIia receptor density.\textsuperscript{73} The presence of the PlA2 allele may be associated with an increase in platelet aggregation after stimulation with
ADP, epinephrine, or collagen and more production of thromboxane A2. In contrast, the homozygous PIa1 genotype appears to be more sensitive to arachidonic acid and thromboxane analogs but not to thrombin or ADP. In clinical studies, as with other polymorphisms, findings have conflicted regarding a significant association between the PI variant and the risk of MI, CAD, cerebrovascular disorders, and arterial or venous thrombosis (Table 4). Even the results of meta-analyses are divided: some have shown no significant link between the PIa2 allele and the risk of MI, CAD, cerebrovascular disease/stroke, or CAD, whereas others have shown slight but significant associations between this polymorphism and the risk of CAD and ischemic coronary events after revascularization.

Mutations in \( \alpha_{IIb}\beta_3 \) and GP Ib are established culprits in inherited disorders of hemostasis. Both were obvious initial candidates to examine associations between genetic variability and thrombosis tendency, yet despite extensive analysis, no clear associations have emerged. Despite the critical and nonredundant nature of these proteins in hemostasis, organisms likely have adapted to tolerate relative small changes in their levels or functions without developing overt thrombosis. In addition, the assays used to detect platelet responsiveness may not be ideally suited to detecting enhanced functions of these proteins. Alternatively, their contribution to platelet phenotypes and clinical outcomes may be very small and require large population analysis to detect. The next section discusses other possible methods for identifying genetic-driven differences in platelet reactions to stimulation.

**GWASs to Identify Genetic Determinants of Platelet Aggregation**

The many benefits of GWASs include the fact that they can be unbiased, identify nonplatelet genes affecting platelet function, provide data on both sequence and copy-number variations, and identify common genetic variants (minor allele frequency >5%) linked to various diseases. However, the results are not always replicable, typically do not identify the genes themselves (most loci identified in GWASs are not located in exon coding regions and thus are not associated
with amino acid changes), and cannot provide information about context or mechanisms. In addition, most variants have been associated with only minor increases in risk, and thousands of subjects are required to identify significant associations with clinical outcomes.

In the classic GWAS, a clinical outcome such as MI is tracked. One method to reduce the need for excessively large samples is to use an intermediate phenotype for analysis. For example, if genes 1 and 2 affect platelet reactivity, it might be more feasible to measure their physiological effects rather than the clinical outcome of MI. This approach requires that the measured variable directly relate to the clinical outcome, and appropriate intermediate phenotypes may not always exist or be readily detectable. With these caveats in mind, several investigations have used this approach to generate provocative and hypothesis-generating findings (Table 5).

Although many of the associations have mapped to proteins of known function in platelets, GWAS have also suggested roles for novel mediators. One example is the platelet endothelial aggregation receptor (PEAR) 1. This type 1 platelet membrane protein undergoes agonist-induced phosphorylation in a GP IIb/IIIa-dependent manner. Herrera-Galeano et al genotyped PEAR1 for 10 single-nucleotide polymorphisms (SNPs) from 1486 healthy people in 2 generations of families with premature CAD enrolled in the GeneSTAR study. The C allele of SNP rs2768759 (A/H11022C), located in the promoter region of the gene, was much more frequent in whites than blacks (70.2% versus 17.7%) and was generally associated in both groups with increased platelet aggregation in response to all agonists at baseline. After aspirin treatment, the associations were stronger and more consistent and remained significant when aggregation was adjusted for baseline responses, consistent with the C allele playing a role in reduced platelet responsiveness to aspirin. The PEAR1 SNP explained up to 6.9% of the locus-specific genetic variance in blacks and up to 2.5% of the genetic variance in whites after aspirin treatment. Thus PEAR1

<table>
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<td>1999</td>
<td>2237 men with CAD*</td>
<td>2.6</td>
</tr>
<tr>
<td>Roest et al</td>
<td>2000</td>
<td>480 women with CV death*</td>
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<td>Cassorelli et al</td>
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<td>Zhao et al</td>
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<td>Zhao et al</td>
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<td>2000</td>
<td>480 women with CV death</td>
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<td>Morita et al</td>
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<td>210 Japanese MI patients</td>
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<td>Rosenberg et al</td>
<td>2002</td>
<td>100 young men with MI</td>
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<tr>
<td>ATV6 et al</td>
<td>2003</td>
<td>1210 young patients with first MI</td>
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<td>Sacchi et al</td>
<td>1999</td>
<td>45 young stroke patients</td>
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<td>Reiner et al</td>
<td>2000</td>
<td>36 young women with stroke</td>
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<td>Cervera et al</td>
<td>2007</td>
<td>82 stroke patients</td>
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<tr>
<td>Carlsson et al</td>
<td>1999</td>
<td>182 stroke patients &gt;50 years old</td>
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<tr>
<td>Corral et al</td>
<td>1999</td>
<td>104 patients with CVD</td>
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<tr>
<td>Iniesta et al</td>
<td>2003</td>
<td>141 patients with primary ICH</td>
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<td>Iniesta et al</td>
<td>2004</td>
<td>103 patients with subarachnoid bleed</td>
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<tr>
<td>Corral et al</td>
<td>1999</td>
<td>101 patients with CAD</td>
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<td>Streifler et al</td>
<td>2001</td>
<td>153 patients with ≥50% carotid stenosis</td>
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<tr>
<td>Azenben et al</td>
<td>2005</td>
<td>171 patients with CAD undergoing CABG</td>
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<td>Jiménez et al</td>
<td>2008</td>
<td>102 patients with SLE</td>
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<td>Carlsson et al</td>
<td>1999</td>
<td>Patients with DVT</td>
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<tr>
<td>Corral et al</td>
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<td>97 patients with DVT</td>
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<td>Hessner et al</td>
<td>1999</td>
<td>233 factor V (Leiden) carriers</td>
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<tr>
<td>Dinauer et al</td>
<td>1999</td>
<td>331 white American VTE patients</td>
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<tr>
<td>Von Beckerath et al</td>
<td>1999</td>
<td>1797 patients undergoing stenting</td>
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Cohort lists numbers of case patients. Data were tabulated in October 2010. APS indicates antiphospholipid syndrome; ATVB, Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group; CABG, coronary artery bypass surgery; CV, cardiovascular; CVD, cerebrovascular disease; DVT, deep vein thrombosis; ICH, intracranial hemorrhage; MACE, major adverse cardiac events; SCD, sudden cardiac death; SLE, systemic lupus erythematosus; VTE, venous thromboembolism.

*Subgroup analysis.

Adapted from Kunicki et al with permission.
appears to play an important role in the response to aspirin in both whites and blacks.

Another variant of the PEAR1 gene, the intron 1 variant (rs12041331A/H11022G), has shown an even stronger association with its expression. The G allele was associated with increased platelet aggregation in response to all agonists, before and after aspirin treatment, in 2076 healthy persons enrolled in GeneSTAR. Frequency of the G allele was 91% in whites and 63% in blacks, and it accounted for up to 3% and 15%, respectively, of the total phenotypic variance in these groups. This SNP is located at a predicted leucine zipper factor binding site (AliBaba2.1), suggesting a potential mechanism for PEAR1 regulation by the variant.

Platelet Expression Profiling

Proteomic and transcriptomic analyses have identified important differences in gene expression, genetic pathways, class predictions/diagnostics, protein phosphorylation patterns, protein interactions, and possible therapeutics targets. Our discussion focuses on gene expression profiling.

Although human platelets are anucleate fragments of megakaryocytes, they retain cytoplasmic mRNA and can translate proteins. Young platelets contain particularly high concentrations of mRNA. Estimates place the number of platelet individual transcripts at 1600 to 3000. Regulation of transcription is enhanced by agonists such as -thrombin, controlled by ligation of integrins such as IIb/3 and IIa/1, and associated with cytoskeletal translocation of eukaryotic translation initiation factor 4E. Initial platelet-profiling studies focused on the use of microarrays and serial amplification of genetic expression evaluations. We focus on data generated in 3 specific contexts: (1) healthy individuals who display differences in platelet aggregation responses, (2) individuals presenting with acute MI, and (3) patients with essential thrombocytosis.

In a recent analysis, platelet RNA was isolated from 288 healthy subjects who had been phenotyped for platelet responsiveness. Gene expression patterns in individuals defined as being hyperreactive (n=18) were compared with those having hyporeactive platelets (n=11). The hyperreactive subjects had 120 upregulated genes and 170 downregulated genes compared with hyporeactive subjects. In particular, expression of genes involved in intracellular signaling and calcium flux differed between the 2 groups.
Hyperreactivity was significantly associated with increased levels of mRNA for vesicle-associated membrane protein 8/endobrevin, a vesicle-soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor required for platelet granule secretion. A vesicle-associated membrane protein 8 SNP (rs1010) has also been associated with platelet reactivity in an age-dependent manner. A role for vesicle-associated membrane protein 8 in platelet reactivity is supported by observations that the rs1010 polymorphism is associated with the risk of MI.124–126

Interpreting the results of transcriptional profiling in acute MI is challenging because changes in gene expression can reflect events triggering or consequences of plaque rupture and thrombosis. Healy et al127 profiled platelet mRNA from patients with acute ST-segment-elevation MI (STEMI, n = 1100) or stable CAD (n = 44), analyzed the transcriptomes, and constructed single-gene models to identify candidate genes with differential expression. Of the 54 differentially expressed transcripts, the most strongly linked to STEMI were CD69 and myeloid-related protein-14 (MRP-14). Plasma levels of MRP-8/14 heterodimer were doubled in patients with STEMI compared with stable CAD (17.0 versus 8.0 μg/mL; \( P < 0.001 \)).

To validate the findings, a prospective, nested, case-control study of 255 pairs of women was conducted within the Women’s Health Study. The risk of nonfatal MI, stroke, or cardiovascular death increased significantly with increasing quartile of MRP-8/14, with women in the highest quartile having a 3.8-fold increase in risk compared with those in the lowest quartile, independent of traditional risk factors or C-reactive protein.127 In another nested case-control study (237 case-control pairs) conducted among patients enrolled in a phase III trial, the median MRP-8/14 level was significantly higher in patients who died or had nonfatal MI at 30 days compared with patients without these events.128 The risk of a repeat cardiovascular event increased with increasing quartile of MRP-8/14 level; patients in the highest quartile had twice the risk of a recurrent event versus patients in the lowest quartile, even after adjusting for standard risk indicators, treatment assignment, and C-reactive protein. Thus, expression of MRP-14 appears to be increased before STEMI, and plasma concentrations of MRP-8/14 might predict the risk of future cardiovascular events in healthy individuals.129

A final example of profiling to identify gene-expression patterns associated with platelet responses is the use of essential thrombocytosis (ET) as a model. Patients with ET have thrombotic complications, hemorrhagic symptoms, or both. Among the first discoveries to emerge from the use of this model were that distinct subtypes of steriodogenic...
17β-hydroxysteroid dehydrogenases are functionally present in human platelets and that their differential expression is associated with ET.\textsuperscript{111} A primary drawback of using ET to model platelet profiling is that it can be difficult to distinguish ET from reactive thrombocytosis. In an attempt to develop class-prediction algorithms, Gnatenko et al studied the platelet transcript profiles of 38 patients with reactive thrombocytosis, 40 patients with ET (24 of whom carried the JAK2V[617]F mutation, a marker of myeloproliferative disorders), and 48 healthy control subjects.\textsuperscript{115} The healthy and ET groups showed little variation by sex (1% of genes differed), but 3% of the genes in the reactive thrombocytosis group were skewed toward men. A subset of 11 biomarker genes was 86.3% accurate in discriminating among the 3 groups, 93.6% accurate in distinguishing between ET and reactive thrombocytosis, and 87.1% accurate in prospective classification of a new group.\textsuperscript{115} In addition, a set of 4 biomarker genes predicted JAK2 wild-type ET in >85% of samples. Genetic biomarker subsets obtained from routine blood sampling might be used to predict thrombocytosis class.

The newest method for platelet profiling involves a multiplex-based platform for simultaneous quantification of platelet transcripts using fluorescent microspheres and intact platelet-rich plasma or gel-filtered platelets lysed in vitro.\textsuperscript{113} With this method, which bypasses the need to isolate RNA, 17 platelet transcripts can be profiled accurately and simultaneously from only 100 μL of whole blood, even for low-abundance platelet transcripts. Results of this method correlate exceptionally well with those from platelet Affymetrix microarrays (r\textsuperscript{2}=0.949; P<0.001) and show no correlation with in-kind–derived leukocyte profiles. This method might be adapted for situations where rapid molecular profiling using whole blood would be valuable.

### Table 4. Correlation Between Presence of Platelet Glycoprotein IIb/IIIa Variant PlA2 and Risk for Adverse Outcomes in Various Thrombotic Disorders

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<th>Cohort</th>
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<td>1999</td>
<td>200 young MI survivors</td>
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<td>374 men with MI</td>
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<td>Gardeman et al\textsuperscript{79}</td>
<td>1998</td>
<td>2252 men with CAD</td>
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<tr>
<td>Joven et al\textsuperscript{80}</td>
<td>1998</td>
<td>250 young men with MI</td>
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<tr>
<td>Anderson et al\textsuperscript{81}</td>
<td>1999</td>
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<td>Cenarro et al\textsuperscript{82}</td>
<td>1999</td>
<td>40 patients with hypercholesterolemia</td>
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<td>Hooper et al\textsuperscript{83}</td>
<td>1999</td>
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<td>Rosenberg et al\textsuperscript{84}</td>
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<td>316 men with MI</td>
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<td>165 women with MI</td>
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<td><strong>Cerebrovascular Disease/Stroke</strong></td>
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<td>Wagner et al\textsuperscript{86}</td>
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<td>Jiménez et al\textsuperscript{84}</td>
<td>2008</td>
<td>102 patients with SLE</td>
<td></td>
</tr>
<tr>
<td>Jiménez et al\textsuperscript{84}</td>
<td>2008</td>
<td>131 patients with APS</td>
<td></td>
</tr>
<tr>
<td><strong>VTE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ridker et al\textsuperscript{78}</td>
<td>1997</td>
<td>121 patients with VTE</td>
<td></td>
</tr>
<tr>
<td>Hooper et al\textsuperscript{83}</td>
<td>1999</td>
<td>91 black patients with VTE</td>
<td></td>
</tr>
<tr>
<td><strong>Restenosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kastrati et al\textsuperscript{91}</td>
<td>1999</td>
<td>1150 patients with stents</td>
<td>1.42</td>
</tr>
</tbody>
</table>

Cohort lists numbers of case patients. Data were tabulated in October 2010. Entries in italics indicate a protective association. APS indicates antiphospholipid syndrome; ICH, intracranial hemorrhage; SAH, subarachnoid hemorrhage. SCD, sudden cardiac death; SLE, systemic lupus erythematosus; VTE, venous thromboembolism.
Although platelet profiling using proteomic/transcriptomic technologies is feasible, several challenges remain, including small amounts of target mRNA, concern for contaminating nonplatelet cells in the preparations, and the challenge of extrapolation to more common platelet disorders and prohibitive costs. To maximize the applicability of profiling methods, consortia must be developed for interinstitutional data exchange and enrollment. Future research should include both pharmacogenomic studies in platelets and comparative pharmacological effectiveness studies by sex and ethnicity.

**Genetic Polymorphisms and the Response to Antiplatelet Therapies**

The use of antiplatelet therapies is a mainstay in the settings of acute coronary syndrome (ACS) and percutaneous coronary intervention (PCI), particularly dual therapy with aspirin and clopidogrel. Recently, genetic variations associated with hyporesponsiveness to antiplatelet therapy have been associated with poorer outcomes. For example, a meta-analysis of 9 studies that collectively enrolled 9684 patients receiving clopidogrel (91% of the patients had undergone PCI, 65% had ACS), 28.5% of patients were carriers of the reduced-function allele of gene CYP2C19. These carriers had a 61% higher risk of a major adverse cardiac event compared with noncarriers. Other studies have linked the presence of CYP2C19 reduced-function variants with greatly increased risks for stent thrombosis with and without cardiac mortality; cardiovascular ischemic events or death; and death, MI, or nonfatal stroke. Moreover, if both CYP2C19 and ABCB1 reduced-function alleles are taken into account, the risk of death is increased by 89% compared with noncarriers.

**Table 5. Genome-Wide Association Studies Related to Platelet Aggregation**

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Variable of Interest</th>
<th>Location of Linkage</th>
<th>Candidate Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans et al</td>
<td>327 monozygotic, 418 dizygotic twin pairs</td>
<td>Platelet count</td>
<td>Chromosome 19, q13.13-13.31</td>
<td>GP VI</td>
</tr>
<tr>
<td>Yang et al 2007</td>
<td>1000 FHS participants from 310 families</td>
<td>ADP-induced PA</td>
<td>rs10493895, chromosome 1</td>
<td>BC064027; DPYD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collagen-induced PA</td>
<td>rs10484128, chromosome 14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epinephrine-induced PA</td>
<td>rs848523, chromosome 2</td>
<td>CRIM1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs565229, chromosome 11</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>rs10506458, chromosome 12</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>rs6811964, chromosome 4</td>
<td>PDGF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs1958208, chromosome 14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs10502583, 18</td>
<td></td>
</tr>
<tr>
<td>Danik et al 2009</td>
<td>17,686 Women’s Genome Health Study participants</td>
<td>Serum fibrinogen level</td>
<td>rs1016988, chromosome 5</td>
<td>SLC22A5, SLC22A4, IRF1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs10479002, chromosome 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs10512597, chromosome 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs1037170, chromosome 17</td>
<td>CD300LF, SLC9A3R1, NAT9</td>
</tr>
<tr>
<td>Trégouët et al 2009</td>
<td>2176 French VTE cases, 2636 French controls</td>
<td>VTE</td>
<td>rs1613662</td>
<td>GP VI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs13146272</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>rs1208134 and rs2420371, chromosome 1</td>
<td>Factor V</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs657152, rs505922, rs630014, chromosome 9</td>
<td>ABO</td>
</tr>
<tr>
<td>Meisinger et al 2009</td>
<td>10,048 subjects, 3 cohorts</td>
<td>Mean platelet volume</td>
<td>rs7961894, chromosome 12</td>
<td>WDR66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs12485738, chromosome 3</td>
<td>ARHGGEF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs2138852, chromosome 17</td>
<td>TAOK1</td>
</tr>
<tr>
<td>Soranzo et al 2009</td>
<td>8586 subjects, 5 cohorts</td>
<td>Mean platelet volume, platelet annexin and fibrinogen binding, P-selectin expression</td>
<td>rs342293, chromosome 7</td>
<td>PIK3CG</td>
</tr>
<tr>
<td>Johnson et al 2010</td>
<td>2753 FHS participants*</td>
<td>PA</td>
<td>7 loci</td>
<td>GP VI, PEAR1, ADRA2A, PIK3CG, JMJD1C, MRH1, SHH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mathias et al 2010</td>
<td>1231 healthy European Americans, 846 healthy black Americans with family history of premature CAD</td>
<td>Epinephrine-, collagen-, ADP-, arachidonic-acid-induced PA; urinary thromboxane B2 level; PFA-100; fibrinogen level; vWF level†</td>
<td>9 loci</td>
<td>MME, PI3-E, GLS3, LDHAL6A</td>
</tr>
</tbody>
</table>

FHS indicates Framingham Heart Study; GeneSTAR, Genetic Study of Aspirin Responsiveness; GP, glycoprotein; KORA, Kooperative Gesundheitsforschung in der Region Augsburg; PA, platelet aggregation; PFA, Platelet Function Analyzer; VTE, venous thromboembolism; vWF, von Willebrand factor.

*Of European ancestry.
†Before and after 14 days of aspirin treatment.
into account, up to half of the ACS population undergoing PCI might have a genotype associated with increased risk of major cardiac events while receiving clopidogrel.\textsuperscript{135}

In May 2009, the US Food and Drug Administration called for addition of information about “poor metabolizers” to the labeling for Plavix (clopidogrel bisulfate).\textsuperscript{136} In March 2010, the agency announced the requirement for a “black-box” warning on the label, specifying that poor metabolizers are at higher risk for cardiovascular events. The labeling defines poor responders as persons who are homozygous for any of the CYP2C19*2 to 18 alleles. The labeling notes that genetic testing can be performed to identify poor responders and that physicians should consider alternative treatment strategies for these persons.\textsuperscript{136} At present, however, the Food and Drug Administration has approved no agent for specific use in poor responders to clopidogrel or in those with a heightened response to the drug.

This issue highlights a conundrum that can stem from improved insight into genetic associations, namely, the lack of a proven therapeutic strategy. For poor responders to clopidogrel, possible strategies include use of a higher dose of clopidogrel or alternate P2Y\textsubscript{12} antagonists, such as prasugrel or ticagrelor, which are newer thienopyridines that depend less on CYP2C19 oxidation for effect and have not been linked to pharmacokinetic or pharmacodynamic differences based on CYP genotype.\textsuperscript{137–139} Small studies have reported improved outcomes with higher doses of clopidogrel when nonresponsiveness was assessed ex vivo, but it is not clear whether these findings will translate to population benefit based on CYP genotype. The study Gauging Responsiveness with a VerifyNow Assay-Impact on Thrombosis and Safety (GRAVITAS, Clinicaltrials.gov #NCT00645918) is currently exploring the use of the VerifyNow test to guide antiplatelet therapy (tailored or standard clopidogrel dosing versus placebo) in 2800 patients undergoing planned stenting, measuring the outcomes of cardiovascular death, nonfatal MI, or definite or probable stent thrombosis within 6 months.\textsuperscript{140} The results of this trial, which may be available in late 2010, should shed light on the value of test-guided antiplatelet therapy. Similar studies will be required to define optimal antiplatelet strategies based on genotype to ensure the best outcomes using a personalized medicine approach.

Conclusions/The Future

Candidate gene-association studies, GWASs, and gene expression profiling continue to reveal novel linkages between polymorphisms in genes coding for platelet function and both thrombotic and hemorrhagic phenotypes. These and ongoing investigations should bring us closer to the day when platelet-directed therapy can truly be individualized according to genomic or transcriptomic characteristics, in addition to endogenous and environmental factors.

Complete knowledge of the relationship between genotype and phenotype is insufficient, however. Alternative management strategies remain to be developed and tested for patients with genotypes linked to platelet hyperresponse, currently the case for clopidogrel and likely to emerge for other antiplatelet agents, as well as platelet hyperresponse.

Appendix: Participants in the 2010 Platelet Colloquium

Bina Ahmed, MD, University of Vermont, Burlington; Dominick J. Angiolillo, MD, PhD, University of Florida College of Medicine, Jacksonville; Wadie F. Bahou, MD, State University of New York, Stony Brook; Diane M. Becker, ScD, and Lewis C. Becker, MD, Johns Hopkins University School of Medicine, Baltimore, MD; Richard C. Becker, MD, Duke Clinical Research Institute, Durham, NC; Paul F. Bray, MD, Thomas Jefferson University, Philadelphia, PA; Pamela B. Conley, PhD, Portola Pharmaceuticals, Inc., South San Francisco, CA; Mary Cushman, MD, MSc, University of Vermont, Colchester; Mitali Das, PhD, Cleveland Clinic, Cleveland, OH; Harold L. Dauerman, MD, University of Vermont College of Medicine, Burlington; Patricia A. French, BS, Left Lane Communications, Chapel Hill, NC; Valentin Fuster, MD, Mount Sinai Medical Center, New York, NY; Haixia Gong, MD, PhD, University of Illinois at Chicago; Brian G. Katona, PharmD, AstraZeneca, Wilmington, DE; Donald Lynch, MD, Johns Hopkins Hospital, Baltimore, MD; Juan Maya, MDAstraZeneca, Wilmington, DE; Leslie V. Parise, PhD, University of North Carolina at Chapel Hill; Jayne Prats, PhD, The Medicines Company, Waltham, MA; Rehan Quyyum, MD, Johns Hopkins Hospital, Baltimore, MD; Christopher P. Rusconi, PhD, Regado Biosciences, Inc., Durham, NC; Marc S. Sabatine, MD, MPH, Brigham and Women’s Hospital, Boston, MA; Daniel I. Simon, MD, Case Western Reserve University School of Medicine, Cleveland, OH; Simona Skjeranke, PharmD, The Medicines Company, Parsippany, NJ; Susan S. Smyth, MD, PhD, University of Kentucky, Lexington; Enrico P. Veltri, MD, Merck Research Laboratories, Kenilworth, NJ; Deepak Voora, MD, Duke University Medical Center, Durham, NC; Tracy Y. Wang, MD, MHS, MSc, Duke Clinical Research Institute, Durham, NC; Ethan J. Weiss, MD, University of California, San Francisco; Marlene S. Williams, MD, The Johns Hopkins University, Baltimore, MD.

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Disclosures

Drs Williams, Weiss, Bahou, and L.C. Becker and P.A. French have no conflicts to disclose. Dr Sabatine has received research grant support from Bristol-Myers Squibb, Sanofi-Aventis, AstraZeneca, and Schering-Plough; received honoraria from Eli Lilly; and consulted for BMS/Sanofi Partnership, Sanofi-Aventis, and Daiichi/Eli Lilly. Dr Simon has received honoraria from BMS/Sanofi Partnership, Daiichi/Eli Lilly, Johnson & Johnson, Portola Pharmaceuticals, Schering Corporation, and The Medicines Company and consulted for BMS/Sanofi Partnership, Daiichi/Eli Lilly, Johnson & Johnson, Portola Pharmaceuticals, Schering Corporation, and The Medicines Company. Dr Parise has received honoraria from SAB, Blood Center, Milwaukee. Dr Dauerman has consulted for BMS/Sanofi Partnership and The Medicines Company. Dr Smyth has received grant support from AstraZeneca, Daiichi/Eli Lilly, Schering Corporation, and The Medicines Company and consulted for BMS/Sanofi Partnership and The Medicines Company.


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for the 2010 Platelet Colloquium Participants

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