Genetic variation of platelet function and pharmacology: An update of current knowledge

Tobias Geisler; Elke Schaeffeler; Meinrad Gawaz; Matthias Schwab
Medizinische Klinik, Innere Medizin III, Kardiologie und Kreislauferkrankungen, Tübingen, Germany

Summary
Platelets are critically involved in atherosclerosis and acute thrombosis. The platelet phenotype shows a wide variability documented by the inherited difference of platelet reactivity, platelet volume and count and function of platelet surface receptors. Several candidate genes have been put into focus and investigated for their functional and prognostic role in healthy individuals and patients with cardiovascular (CV) disease treated with antiplatelet agents. In addition to genetic variation, other clinical, disease-related and demographic factors are important so-called non-genetic factors. Due to the small effect sizes of single nucleotide polymorphisms (SNP) in candidate genes and due to the low allele frequencies of functional relevant candidate SNPs, the identification of genetic risk factors with high predictive values generally depends on the sample size of study cohorts. In the post-genome era new array and bioinformatic technologies facilitate high throughput genome-wide association studies (GWAS) for the identification of novel candidate genes in large cardiovascular cohorts. One of the crucial aspects of platelet genomic studies is the precise definition of a specific clinical phenotype (e.g. stent thrombosis) as this will impact importantly the findings of genomic studies like GWAS. Here, we provide an update on genetic variation of platelet receptors and drug metabolising enzymes under specific consideration of data derived by GWAS. The potential impact of this information and the role in personalised therapeutic concepts will be discussed.

Keywords
Platelet physiology, platelet pharmacology, polymorphisms

Introduction
Platelets are critically involved in atherosclerosis and acute thrombosis leading to major cardiovascular ischaemic events. The platelet phenotype shows a wide variability documented by the inherited difference of platelet reactivity, platelet volume, platelet count and function of platelet surface receptors.

Several candidate genes have been put into focus and extensively investigated for their functional and prognostic role in healthy individuals and patients with cardiovascular disease treated with antiplatelet agents. In addition to genetic variation, other factors (e.g. age, sex, weight, body fat, alcohol consumption, concomitant drugs, nutritional status, cardiovascular, liver and renal function, environmental pollutants) are important so-called non-genetic factors possibly also altering platelet function and antiplatelet drug response (1). Due to the small effect sizes of single nucleotide polymorphisms (SNP) in candidate genes and due to the low allele frequencies of functional relevant candidate SNPs, the identification of genetic risk factors with high predictive values generally depends on the sample size of study cohorts including index patients and controls. In the post-genome era new array and bioinformatic technologies facilitate high throughput genome-wide association studies (GWAS) for the identification of novel candidate genes in large cardiovascular cohorts (2, 3). One of the crucial aspects of platelet genomic studies is the precise definition of a specific clinical phenotype (e.g. stent thrombosis, angiographically verified coronary thrombosis) as this will impact importantly the findings of genomic studies like GWAS as well as next generation sequencing (NGS) studies. Phenotypes that are strongly linked to cardiovascular and platelet associated outcome have been suggested to be a reasonable approach. Here, we provide an update on genetic variation of platelet receptors and drug metabolising enzymes under specific consideration of data derived by genome-wide approaches. The potential functional impact of this information and the role in personalised therapeutic concepts will be discussed.

Genetic inheritance of platelet function
To date, most strategies to elucidate the impact of genetic variation on platelet reactivity relied on candidate gene approaches and used
single or only few tests to assess platelet function as primary outcome variable. Some of these observations have not been replicated or were not confirmed by functional genomic approaches. A major prerequisite in platelet genomics is the selection of the appropriate phenotype which is most critical. Candidate gene analyses have major shortcomings, in particular in platelet genomics. First, the majority of platelet SNPs shows a very small prevalence with minor allele frequency of less than 0.2 (Table 1). Second, most platelet phenotypes have only a small effect size with a high inter-individual variability influenced by non-genetic traits. Thus, large cohorts and genomic technologies are required to detect rare variants and to elucidate their effects with a sufficient statistical power in multivariate analyses considering multiple testing as well. Previous candidate gene analyses have mainly focussed on genes considered to be important in the function of platelets and haemostatic disorders. In particular, genes have been investigated that encode for platelet surface receptors involved in platelet adhesion via glycoprotein (GP)V1 (4, 5), α2β1 integrin (6) or vWF-GPIbα (7, 8), in fibrinogen-dependent platelet aggregation via αIIbβ3 integrin (9, 10) or in platelet signalling induced by other agonists (11, 23). Candidate gene studies investigating the impact of single SNPs on platelet function parameters are summarised in Table 1. Functional genomic approaches including much larger number of candidate genes have advanced the field thus bridging the gap between limited candidate gene studies and genome-wide association studies. Thus, Jones et al. (48) recently described 17 independently associated SNPs from a pool of 1,327 SNPs from 97 preselected gene regions accounting for 48% of the variation in platelet reactivity induced by either cross-linked collagen related peptide (CRP-XL) or ADP. Of note, they found a strong influence of GPV1 polymorphic variant rs1613662 quantitative trait locus (QTL) for platelet response to CRP-XL. In the first meta-analysis of GWA studies investigating the effects of genetic variants on agonist-induced platelet aggregation in a cohort of 4,000 individuals of European ancestry, seven loci were identified to be significantly associated with platelet aggregation (Table 2). The two most strongly associated variants were located near or within the genes encoding for GP6 and platelet endothelial aggregation receptor 1 (PEAR1) (49).

Some platelet physiologic parameters are highly heritable and influence platelet function and outcome. Mean platelet volume (MPV) and platelet count have a genetic background. They correlate with agonist-induced platelet function and αIIbβ3 expression and have been suggested as independent cardiovascular risk factors (12-15). Mean platelet volume is associated with megakaryopoiesis and beta-thromboglobulin release (16). Different genetic variants have been associated with MPV and platelet count in candidate gene analyses. Whole genome expression analysis revealed highly significant association of the SNP rs342293 located on chromosome 7q22.3 with mean platelet volume in healthy subjects (17). To this end, Gieger et al. have performed the largest meta-analysis of GWAS so far, including approximately 67,000 individuals with European background. They found 68 independent genomic regions that were related to MPV and platelet count (25 and 43 regions for MPV and platelet count, respectively) (18). The 68 reported loci explain about 5% of variability of platelet count and 10% of variability of MPV. Replication of selected loci was obtained in cohorts with different ethnic background (19, 20).

Genetic variants of specific platelet receptors

ADP receptors

The adenosine diphosphate receptor on blood platelets is formed by different subunits. Among them P2Y1 and P2Y12 play the major role for ADP mediated platelet aggregation. There is marked individual variability measured by ADP induced platelet aggregation and fibrinogen binding in healthy volunteers and patients. Previously, different polymorphic variants have been identified in the genetic region encoding for both subunits located on chromosome 3. Five different genetic polymorphisms for the P2Y12 gene have been reported by Fontana et al. Of those, four were in strong linkage disequilibrium. Thus, two haplotypes H1 and H2 were described, which occur with frequencies of 86% (H1) and 14% (H2), respectively. In case control studies the H2 haplotype was associated with increased prevalence of coronary and peripheral artery disease (CAD, PAD) (21, 22). The functional prognostic role of the P2Y12 H2 haplotype is still unclear. More recently, Hetherington identified five polymorphisms in the P2Y1 gene and 11 in the P2Y12 gene (Table 1) (23). Of note, none of the previously described polymorphism, but the rs1472122 polymorphic variant was associated with platelet function in a genome-wide approach (48).

Collagen receptors

Collagen plays a crucial role in platelet adhesion and activation via binding of two major platelet surface receptors: the integrin α2β1 (GPla-IIa) and the platelet specific receptor GPVI. Integrin α2 (ITGA2, CD4b) subunit forms a heterodimer with integrin β1 subunit, thus acting as cellular collagen receptor. Three major alleles encoding for the α1 chain have been previously described. An association with increased expression of α2β1 has been reported for allele 1 (807T/1648G/2531C), whereas the other two alleles (allele 2: 807C/1648G/2531C + allele 3: 807C/1648A/2531C) have been associated with decreased receptor expression (24). The 807T allele (high receptor density) was initially described to be associated with the risk for acute myocardial infarction (MI) in case-control studies (25, 26). There are no relevant data on the functional impact of any known polymorphic variant encoding for the β1 subunit (ITGB1). GPVI is the second main collagen receptor on platelets. It is a member of the IgG superfamily which is involved in platelet activation via activation of phospholipase C2 and tyrosine phosphorylation. Despite being related to decreased receptor expression and collagen-signalling (27), the GPVI I3254CC genotype has been associated with increased risk of MI and thrombus formation (28, 29). In genome-wide functional approaches, the variants rs1613662 and rs1671152 have been associated with agonist-induced P-selectin expression, fibrinogen receptor binding and...
Platelets: basic mechanisms and translational implications

Collagen receptors

GPVI
Platelet adhesion
rs1613662 (13254T>C)
r
0.16
rs1671152
0.14
36, 37, 52

GPIBA
Platelet adhesion
rs2243093 (-5T/C, Kozak sequence polymorphism)
r
0.18
rs6065
0.10
8

ITGA2
Platelet adhesion
rs1126643 (807C>T)
r
0.38
rs28095 (52C>T)
r
0.36
rs1801106 (1648G>A)
r
0.08
rs41305896
0.03
39-42

Fibrinogen receptor

ITGAXB
Platelet aggregation
rs5911
0.41
43

ITGB3
Platelet aggregation
rs5918 (196T>C, HPA-1a/b, PIa1/A2)
r
0.15
44

Other platelet surface receptors

TBX2A2R
Thromboxane A2 receptor (TP)
Platelet activation
rs1131882 (795T>C)
r
0.13
rs4523
0.30
rs5758
0.45
11

F2R
Protease-activated receptor (PAR) 1
Thrombin mediated platelet activation
rs168753 (IVSn-14 A>T)
r
0.14
rs11267092 (S61 I/D)
0.14
45, 90

α-Adrenergic receptor 2 A (ADRA2A)
Shear-mediated platelet activation
rs553668 (1780G>A)
r
0.41
46

Immunoglobulin-γ FC receptor IIa (FCGR2A)
Platelet activation
rs1801274 (H131R)
r
0.43
47

Fibrinogen-receptor

ITGB3 is coded by ITGB3, encompassing 15 exons on chromosome 17q21.32. The most intensely studied polymorphic variant is SNPs rs5918. This belongs to the platelet alloantigen systems HPA-1a/b (PIa1/A2). There are conflicting data about the impact on innate platelet reactivity and prognostic role of the HPA-1b polymorphism in cardiovascular diseases. Possible reasons are the sample size of previous candidate gene studies with respect to low minor allele frequency and possible different effects regarding to the selected platelet function phenotype (30) and testing protocol (31). Whereas initial studies with insufficient cohort sizes suggested that ITGB3 gene A1/A2 polymorphism was associated with the risk for developing CAD and coronary/cerebrovascular thrombosis (32, 78), subsequent meta-analyses with combined cohort sizes have not confirmed these assumptions (33, 34).

Table 1: Overview over candidate genetic variants of platelet receptors associated with innate platelet reactivity.

<table>
<thead>
<tr>
<th>Platelet receptor gene</th>
<th>Function</th>
<th>SNP, ID</th>
<th>Minor allele frequency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP receptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2RY1</td>
<td>Platelet aggregation</td>
<td>rs3755711 (190 G&gt;C)</td>
<td>0.15</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1065776 (893 C&gt;T)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs701265 (1622A&gt;G)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>P2RY12</td>
<td>Platelet aggregation</td>
<td>rs3821667 (145 C&gt;T)</td>
<td>0.17</td>
<td>22, 23, 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2046934 (IntB742T&gt;C, tag SNP for H2 haplotype)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs5853517 (IntB798-A)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs138626 (IntB1209T-C)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs6785930 (234C&gt;T)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs6809699 (52G&gt;T)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Collagen receptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPVI</td>
<td>Platelet adhesion</td>
<td>rs1613662 (13254T&gt;C)</td>
<td>0.16</td>
<td>36, 37, 52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1671152</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>GPIBA</td>
<td>Platelet adhesion</td>
<td>rs2243093 (-5T/C, Kozak sequence polymorphism)</td>
<td>0.18</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs6065</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>ITGA2</td>
<td>Platelet adhesion</td>
<td>rs1126643 (807C&gt;T)</td>
<td>0.38</td>
<td>39-42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs28095 (52C&gt;T)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1801106 (1648G&gt;A)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs41305896</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITGAXB</td>
<td>Platelet aggregation</td>
<td>rs5911</td>
<td>0.41</td>
<td>43</td>
</tr>
<tr>
<td>ITGB3</td>
<td>Platelet aggregation</td>
<td>rs5918 (196T&gt;C, HPA-1a/b, PIa1/A2)</td>
<td>0.15</td>
<td>44</td>
</tr>
<tr>
<td>Other platelet surface receptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBX2A2R</td>
<td>Platelet activation</td>
<td>rs1131882 (795T&gt;C)</td>
<td>0.13</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4523</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs5758</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>F2R</td>
<td>Thrombin mediated platelet activation</td>
<td>rs168753 (IVSn-14 A&gt;T)</td>
<td>0.14</td>
<td>45, 90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs11267092 (S61 I/D)</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>α-Adrenergic receptor 2 A (ADRA2A)</td>
<td>Shear-mediated platelet activation</td>
<td>rs553668 (1780G&gt;A)</td>
<td>0.41</td>
<td>46</td>
</tr>
<tr>
<td>Immunglobulin-γ FC receptor IIa (FCGR2A)</td>
<td>Platelet activation</td>
<td>rs1801274 (H131R)</td>
<td>0.43</td>
<td>47</td>
</tr>
</tbody>
</table>
Pharmacogenetics and genomics of antiplatelet therapy

Basically, pharmacogenetics or pharmacogenomics (62, 63) investigates underlying mechanisms for differences in drug response with major focus on genetic variation. While pharmacogenetics addresses particularly the sequence of a single gene (or more than one gene) with consequences on gene expression and function related to drug response and/or adverse drug reactions (ADR), pharmacogenomics focuses on a shift from a candidate gene approach to the elucidation of multiple genes, gene-gene interactions, and other genome-derived mechanisms including a whole array of different technologies commonly termed as –omics technologies (64). Thus, pharmacogenomics offers the possibility to identify novel drug targets as part of the drug development process (65).

Table 2: Overview over candidate genes identified in genome wide association studies and functional genomic approaches with impact on platelet function phenotype and/or clinical outcome. PSADP: adenosine-diphosphate (ADP) induced P-Selectin expression; FADP: ADP induced fibrinogen binding; PCRPXL: CRP-XL induced P-Selectin expression, FCRPXL: CRP-XL induced fibrinogen binding; PA: Agonist induced platelet aggregation; SNP: single nucleotide polymorphism, according to (46, 47).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>SNP</th>
<th>Functional phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPVI</td>
<td>Collagen-induced activation/signalling</td>
<td>rs1613662</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1671152</td>
<td>+</td>
<td>49</td>
</tr>
<tr>
<td>PEAR1</td>
<td>Platelet-contact induced activation</td>
<td>rs11264579</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3737224</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs12566888</td>
<td>+</td>
<td>49</td>
</tr>
<tr>
<td>JAK2</td>
<td>Complex signalling pathways and effects on platelet function, Thrombopoetin signalling pathway, thrombocytosis</td>
<td>rs10429491</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>P2RY12</td>
<td>ADP-induced activation/signalling</td>
<td>rs1472122</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>RAF1</td>
<td>cell proliferation, cAMP dependent platelet activation</td>
<td>rs3729931</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>FCER1G</td>
<td>collagen-mediated platelet activation</td>
<td>rs3557</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>GNAZ</td>
<td>Guanine nucleotide-binding protein alpha; cAMP dependent platelet activation by stimulation of adenyl cyclase</td>
<td>rs3788337</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>VAV3</td>
<td>collagen-mediated platelet activation</td>
<td>rs17229705</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>CD36</td>
<td>Leukocyte differentiation antigen, receptor for thrombospondin (GPV) on platelets</td>
<td>rs1049654</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>MAP2K2</td>
<td>Involved in Mitogen-activated protein kinase pathway</td>
<td>rs350916</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>MAPK14</td>
<td></td>
<td>rs851007</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>MAP2K4</td>
<td></td>
<td>rs41307923</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>ITGA2</td>
<td>Cell Adhesion, collagen binding, Integrin mediated signaling pathway</td>
<td>rs41305896</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>AKT2</td>
<td>Platelet secretory pathways, release of dense and alpha granule contents</td>
<td>rs41275750</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>ITPR1</td>
<td>Intracellular calcium release</td>
<td>rs17786144</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>ADRA2A</td>
<td>Alpha2A adrenergic receptor; epinephrine induced platelet aggregation</td>
<td>rs4311994</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>MIRV1</td>
<td>Regulation of intracellular calcium</td>
<td>rs7940646</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>SHH</td>
<td>Sonic hedgehog, function unknown</td>
<td>rs2363910</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>PIK3CG</td>
<td>Phosphatidylinositol 3-kinase, PI3K signalling pathway</td>
<td>rs342286</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>JMD1C</td>
<td>Jumonji domain-containing protein 1C, function in platelets unknown</td>
<td>rs10761741</td>
<td>+</td>
<td>48</td>
</tr>
</tbody>
</table>

© Schattauer 2013
The following part will focus on specific aspects of antiplatelet drugs frequently used and introduced since several years as well as briefly on novel antiplatelet drugs with so far limited data regarding pharmacogenomics issues.

**Aspirin**

Polymorphisms of cyclooxygenase 1 (COX-1) are rare and evidence about real effects regarding aspirin responsiveness and prognostic efficacy is weak due to small size of individual studies (66-69). Of note, a number of studies suggested that aspirin responsiveness may be independent of both COX-1 and COX-2 thus limiting the possible impact of polymorphic variants in the COX gene (70, 71). Moreover, genetic variants of activation pathways indirectly related to COX-1 influencing platelet activation and aggregation have been previously reported to impair aspirin effects (72).

The HPA-1 polymorphism (HPA-1a/b, PIA1/A2, rs5918) of the platelet GPIIIa subunit (integrin beta3) has been initially associated with response to antiaggregatory medication, but there is still an ongoing controversial discussion about the particular allele influencing response to aspirin. Whereas some studies observed reduced response to aspirin in HPA-1b allele carriers (73-75),

**Table 3: Genetic variants involving antiplatelet drug metabolism and efficacy.**

<table>
<thead>
<tr>
<th>Antipatelet drug</th>
<th>Gene</th>
<th>SNP</th>
<th>Response to antiplatelet therapy / effect on clinical outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>PEAR1</td>
<td>rs12041331, rs12566888, rs2644604, rs2768759</td>
<td>Higher platelet aggregation under aspirin treatment</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>P2RY12</td>
<td>rs7615865, rs1388623, rs1388622, rs7634096, rs7637803</td>
<td>Lower platelet aggregation under aspirin treatment</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>P2RY1</td>
<td>rs1439010, rs1371097, rs701265, rs12497578, rs2312285</td>
<td>Association with serum thromboxane B2 levels under aspirin treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITGB3</td>
<td>rs5918 (196T&gt;C, HPA-1a/b, PIA1/A2)</td>
<td>Controversial data regarding impact on sensitivity to aspirin</td>
<td>73-77</td>
</tr>
<tr>
<td></td>
<td>TXBA2R</td>
<td>rs1131182</td>
<td>Higher platelet reactivity under aspirin treatment in diabetic patients</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>ADRA2A</td>
<td>rs4311994</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLA2G7</td>
<td>rs7756935</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9p21.3</td>
<td>rs10120688</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>CYP2C19*2</td>
<td>rs4244285</td>
<td>Loss-Of-Function, reduced active metabolite levels, reduced inhibition of aggregation and worse cardiovascular outcome in PCI patients</td>
<td>94-100</td>
</tr>
<tr>
<td></td>
<td>CYP2C19*3</td>
<td>rs4986893</td>
<td>Gain-of-Function, enhanced platelet aggregation inhibition, increased bleeding rates, controversial data on effect size</td>
<td>53, 54</td>
</tr>
<tr>
<td></td>
<td>CYP2C19*17</td>
<td>rs12248560</td>
<td>Majority of studies suggest no significant effect on clopidogrel metabolite levels and clopidogrel dependent aggregation inhibition</td>
<td>55-59</td>
</tr>
<tr>
<td></td>
<td>PON1</td>
<td>rs662 (192Q&gt;R)</td>
<td>Greater levels of clopidogrel in allele carriers</td>
<td>106-112</td>
</tr>
<tr>
<td></td>
<td>CES1</td>
<td>rs71647871 (143G&gt;E), rs2244613</td>
<td>Homozygous allele carriers have reduced bioavailability of clopidogrel and higher risk for cardiovascular events</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>ABCB1/MDR1</td>
<td>rs1045642 (3435 C&gt;T), rs1128503 (1236C&gt;T), rs2032582 (2677T&gt;A)</td>
<td>Majority of studies suggest no significant effect on clopidogrel metabolite levels and clopidogrel dependent aggregation inhibition</td>
<td>61, 122</td>
</tr>
<tr>
<td>Prasugrel</td>
<td>CYP2C19*2</td>
<td>rs4244285</td>
<td>Possible effect on platelet reactivity and bleeding risk in prasugrel treated patients</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>CYP2C19*17</td>
<td>rs12248560</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ticagrelor</td>
<td>No significant genotype-phenotype association described</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombin receptor antagonists</td>
<td>No significant genotype-phenotype association described</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thrombosis and Haemostasis 110.5/2013 © Schattauer 2013
others claimed increased sensitivity (76, 77). The prognostic role of the PIA polymorphism in aspirin-treated cardiovascular patients has also been controversially discussed. In a pilot case–control study including 71 patients and 68 controls, Weiss et al. (78) found an association between the HPA-1b allele and the risk for acute coronary syndromes (ACS). In contrast, candidate gene analyses from the Physicians-Health study did not show relevant differences in the frequency distribution of this allele in 374 patients with a history of MI compared with 704 matched controls (79). Subsequent clinical trials could not confirm a significant impact of this polymorphism on clinical outcome in patients with symptomatic CAD treated with aspirin (80, 81). In another candidate gene study, four SNPs were significantly associated with increased platelet reactivity measured by Platelet-Function-Analyzer (PFA)-100 in diabetic patients treated with aspirin (52).

Platelet endothelial aggregation receptor 1 (PEAR1) is thought to be important in platelet contact-induced activation (82). Recently, functional genomic and genome-wide association approaches have identified its role for agonist induced platelet activation and aggregation in healthy subject (48, 49). In a candidate gene study the C allele of rs2768759 [A/C] located in the promoter region of the gene was associated with lower responsiveness to aspirin measured by collagen and epinephrine-induced platelet aggregation in 1,486 healthy individuals being treated with aspirin (83). Recently, a common variant rs12041331 in intron 1 has been found to account for up to 15% of variation of platelet function (84). Recently, a common variant rs12041331 in intron 1 has been found to account for up to 15% of variation of platelet function measured by collagen and epinephrine-induced ADP (85) at baseline and during aspirin treatment (84) (Table 3).

### Clopidogrel

Genetic variation of platelet receptors involved in platelet adhesion and aggregation have been investigated concerning their role on clopidogrel pharmacodynamic and clinical efficacy. Although some case-control studies reported an association of the H2 haplotype of the P2Y12 receptor gene and clopidogrel response (85), the majority of studies failed to demonstrate a significant impact on inhibition of platelet aggregation or prognostic impact in clopidogrel treated patients (86–89). Intervening sequence-14 A/T polymorphism in the gene region encoding for PAR1 was associated with diminished clopidogrel effects on aggregation inhibition and procoagulant activity in PCI patients (90); however, this was not confirmed in another study in patients with stable CAD undergoing elective PCI (91).

Variability of clopidogrel response is associated with genetic variance in CYP450 enzyme expression and activity, predominantly by CYP2C19. Briefly, in a two-step enzymatic conversion clopidogrel is metabolised into a biologically active thiol metabolite. The first step, a monoxygenation reaction of the thiophene ring by CYP450 enzymes (i.e. CYP1A2, CYP2B6, CYP2C19) leads to 2-oxo-clopidogrel, a thiolactone metabolite. The second step results in a CYP450-dependent (i.e. CYP2B6, CYP2C9, CYP2C19, CYP3A4/5) oxidative opening of the thiolactone ring of 2-oxo-clopidogrel (92). CYP2C19 is highly genetically polymorphic, with alleles that are associated with both reduced and enhanced metabolic capacity (http://www.cypalleles.ki.se/cyp2c19.htm). These polymorphisms include the loss-of-function alleles CYP2C19 *2, *3, *4 and *5 and the ultra-rapid metaboliser allele CYP2C19*17 (93). Subjects carrying the reference sequence homozygously (CYP2C19*1/*1) are termed as extensive metaboliser (EM) who are efficiently able to convert clopidogrel into the active metabolite with highest levels of platelet inhibition following clopidogrel exposure. These results are in line with a number of studies clearly demonstrating that in contrast to EM patients individuals who are carrying the loss-of-function allele CYP2C19*2 in a homozygous (*2/*2) or compound heterozygous manner (e.g. *2/*3), result in a poor metaboliser (PM) status and have significantly lower levels of platelet aggregation inhibition following clopidogrel administration.

A series of recent articles have explored the effect of genetic polymorphisms in CYP2C19 on the anti-platelet activity of clopidogrel as well as clinical outcomes following PCI. Candidate gene association studies, investigating platelet function phenotypes, found a significant impact of CYP2C19 PM patients on higher platelet aggregation in clopidogrel treated patients (94, 95). Subsequent studies investigated the impact of CYP2C19*2 and cardiovascular outcome. For instance, Simon et al. examined 2,208 patients that presented with acute MI who received clopidogrel therapy and showed that patients who carried one or more CYP2C19 loss-of-function allele(s) have had higher rates of cardiovascular events than non-carriers (96). The risk of death, non-fatal MI or cardiovascular stroke at one year after PCI was 3.58 times higher in patients carrying two loss-of-function alleles compared to wild-type patients. Trenk et al. associated enhanced platelet activity and adverse clinical outcomes in patients with the CYP2C19*2 allele in 797 patients followed for one year after PCI (97). A substudy published by Mega et al. examined the association between CYP2C19 genotypes and cardiovascular outcome in a subset of 1,477 clopidogrel-treated patients enrolled in the TRITON-TIMI 38 study (98). This study demonstrated that carriers of at least one loss-of-function CYP2C19 allele had a 53% increase in the composite primary efficacy endpoint (i.e. death from cardiovascular causes, myocardial infarction, or stroke) compared with non-carriers (12.1% vs 8.0%, p=0.01) over 15 months of follow-up. This initial finding from a randomised clinical trial (RCT) was followed further by retrospective analyses of three other large RCTs comparing clopidogrel against active comparator or placebo for different indications, the CURE, the ACTIVE-W and the CHARISMA trial. In the CURE trial, clopidogrel was compared against placebo in patients with ACS either undergoing PCI or being medically managed. A total of 5,059 patients were genotyped for single-nucleotide polymorphisms (*2, *3, *17). Clopidogrel reduced the rate of the primary efficacy outcome similarly in patients who were heterozygous or homozygous for loss-of-function alleles compared to non-carriers of the alleles. In contrast, gain-of-function carriers (i.e. patients carrying the CYP2C19*17 allele) have
had a significant benefit from clopidogrel treatment as compared with controls. The subgroup analysis from the ACTIVE-W trial published in the same paper showed that among 1,156 genotyped patients, there was no interaction regarding efficacy or bleeding between the treatment arms and the metaboliser phenotype (loss-of-function carrier status, or gain-of-function carrier status) (99). In the CHARISMA trial clopidogrel was compared to placebo in patients with established stable cardiovascular disease or with multiple atherothrombotic risk factors. A total of 4,819 patients were genotyped. The rates of ischaemic and bleeding events were compared between carriers and non-carriers of loss-of-function (CYP2C19*2,*3) and gain-of-function (CYP2C19*17) alleles in patients randomised to clopidogrel vs placebo. No association was found between CYP2C19 status and ischaemic outcomes in patients treated with clopidogrel. Bleeding occurred significantly less in variant carriers for loss of function alleles compared to non-carriers (100). The currently only available GWA study so far was performed by Shuldiner et al. (3). The PAPI (Amish Pharmacogenomics of Anti-Platelet Interventions) study was performed in a genetically well defined cohort of 429 Amish healthy volunteers. Of note, the authors found that indeed SNPs on chromosome 10q24 within the CYP2C18–CYP2C19–CYP2C9–CYP2C8 gene cluster were strongly associated with decreased clopidogrel response. The rs12777823 variant was nearly in complete linkage disequilibrium with the CYP2C19*2 variant, thus accounting for up to 12% of variability of on-treatment platelet aggregation. Although there is still a major need for the identification of additional factors substantially contributing to the variability of clopidogrel associated platelet aggregation, the study by Shuldiner et al. conclusively confirms the high potential of GWA trials to identify biological meaningful genes as previously shown for warfarin and its association to polymorphisms in CYP2C9 and VKROC1 (101-103). Further GWA studies including larger patient cohorts are currently underway, based on the initiative of the international clopidogrel consortium (http://www.pharmgkb.org/page/icpc), to identify additional genes and rare genetic variants that determine clopidogrel response variability.

In addition to the results of individual studies, recently two meta-analyses (104, 105) focused on the prognostic impact of CYP2C19 polymorphisms with regard to clopidogrel treatment. These meta-analyses came to different conclusions - whereas the first confirmed a significant impact of CYP2C19 loss-of-function genotypes on cardiovascular outcome, the second did not. Of note, these two analyses not only differed in the number and size of included trials, but more importantly in the heterogeneity of the included trials. In most individual trials encompassed by the meta-analysis by Mega et al. homogenous collectives of PCI patients were included, whereas the latter meta-analysis included also trials with patients medically managed due to other reasons. The size of PCI trial vs none-PCI trials was significantly different (small size PCI trials vs larger size none-PCI trials) suggesting an important selection bias. Of note in the meta-analysis by Holmes et al. stent related events, i.e. stent thrombosis, were significantly associated, suggesting that the CYP2C19 loss-of-function genotype has a prognostic role mainly in cohorts of clopidogrel-treated patients undergoing PCI.

Clopidogrel bioactivation involves two sequential steps of oxidation and hydrolysis by plasma esterases. Paraoxonase (PON) is an arylesterase involved in clopidogrel bioactivation. The latter step of hydrolysis is mediated by PON1 and PON3. Initially, Taubert et al. suggested an effect of PON1 polymorphism on active metabolite levels, decreased clopidogrel-dependent platelet aggregation inhibition and cardiovascular outcome in patients undergoing PCI (106). However, several other studies could neither confirm a significant effect of this polymorphism on active metabolite levels, nor on platelet function phenotype and cardiovascular outcome in clopidogrel treated healthy volunteers and CAD patients (107-111). Conclusively, a recent meta-analysis on the existing studies investigating relationship of PON1 and clopidogrel response found no significant association (112). The discrepancy may be explained by recent systematic investigations of the metabolism of 2-oxo-clopidogrel using human liver microsomes and human patient samples (113, 114). For the opening of the thiolactone ring of 2-oxo-clopidogrel a P450-dependent redox bioactivation of 2-oxo-clopidogrel leads to the formation of the 4b cis isomer. The PON-1 catalysed hydrolysis of 2-oxo-clopidogrel results in the formation of a minor thiol metabolite isomer, the 4b "endo" thiol isomer. Dansette et al. (113, 114) clearly showed that the major bioactive thiol isomer detected in the plasma of clopidogrel-treated patients is the CYP450-dependent 4b cis-thiol diastereoisomer. Moreover the most active thiol isomers toward the platelet P2Y12 receptor are the cis- but not the trans-thiol diastereoisomers (115). Thus, probably misleading results by Bouman et al. regarding the major impact of PON-1 on clopidogrel metabolism and outcome may be based on a non-specific method for the separation between different cis- and trans-thiol metabolite isomers whereas Dansette et al. used a novel highly sensitive HPLC-mass spectrometry method (114).

Another enzyme involved in the drug metabolism of clopidogrel is the carboxylesterase 1 (CES-1) only recently put in focus of clopidogrel pharmacogenomics. The serine protease is primarily responsible for the conversion of clopidogrel, its intermediate 2-oxo-clopidogrel, and the final bioactive thiol metabolite into biologically inactive carboxylic acid derivatives. The G143E genetic variant in the region of CES-1 has been found significantly linked to enzyme function. Recently, Lewis et al. carried out an association study on 566 patients enrolled in the PAPI study and found a significant association of the CES-1 143E SNP allele with higher active metabolite levels and higher inhibition of ADP-induced platelet aggregation (60) (Table 3). Of note, there is first evidence that CES1 genetic variants may alter pharmacokinetic parameters of drug agents as recently shown for oseltamivir (116). Regarding clopidogrel treatment additional studies are required to elucidate more precisely the impact of CES on clinical outcome in addition to CYP2C19 polymorphism.
**Prasugrel**

In contrast to clopidogrel, CYP450 enzymes are involved only in one step of prasugrel metabolism with a minor contribution of CYP2C19. Additionally CYP3A4/5, CYP2B6, and CYP2C9 enzymes are involved in the bioactivation of prasugrel with potential pharmacogenetic implications (117, 118). Plasma levels of the active metabolite were not influence by common genetic variants of CYP2C19, CYP2C9, CYP2B6, CYP3A5 or CYP1A2 (98). A first study indicated that clinical efficacy of prasugrel may not be affected by CYP2C19*2 in subcohort of the TRITON-TIMI-38 study population (98). It has been suggested that prasugrel treatment might overcome high on-treatment platelet reactivity compared to high dose (150 mg daily) clopidogrel treatment in CYP2C19*2 allele carriers (119). However, very recently clinical studies report on a significant impact of CYP2C19 polymorphisms (*2 and *17) on platelet reactivity assessed by the vasodilator-stimulated phosphoprotein (VASP) platelet aggregation in patients treated with prasugrel after ACS. Moreover, the bleeding risk in chronic treatment by prasugrel was influenced by CYP2C19 genetics (120). In addition, another study indicates that both clopidogrel and prasugrel therapy may be altered by the CYP2C19 *2 and *17 alleles (121). Generally, prasugrel appears to be superior to clopidogrel treatment regarding platelet inhibition. Nevertheless further studies, however, are needed to conclusively clarify the contribution of genetic variation in CYP450 enzymes involved in prasugrel bioactivation and clinical outcome. Regarding the ATP-dependent efflux membrane transporter ABCB1 encoding for platelet GP (multidrug resistance protein [V ASP]) phosphorylation and VerifyNow P2Y12 assays) as a significant impact of another study indicates that both clopidogrel and prasugrel therapy may be mediated by microsomal CES (128) with potential impact of genetic variation in CES genes (129).

**Ticagrelor**

Ticagrelor is primarily metabolised by CYP3A4/5 enzymes (123), and CYP2C19 does not seem to play a relevant role in drug metabolism. Nevertheless, in a first study genotyping for CYP2C19 alleles (*2, *3, *4, *5, *6, *7, *8, *17) was performed in CAD patients treated with ticagrelor (180-mg load, 90 mg BID) (n=92) vs clopidogrel (600-mg load, 75 mg/day) (n=82) (124). As expected, the pharmacodynamic antiplatelet effect of ticagrelor was irrespective of CYP2C19 genotypes. In addition in the PLATO trial a total of 10,285 from the original enrolled 18,624 patients were genotyped for CYP2C19 loss-of-function alleles (*2, *3, *4, *5, *6, *7, and *8). Regarding the primary outcome (cardiovascular death, MI, or stroke) ticagrelor was superior to clopidogrel, irrespective of CYP2C19 genotypes. The primary outcome rate was 8.6% vs 11.2% in patients with any CYP2C19 loss-of-function genotypes (p=0.038) as well as 8.8% vs 10.0% in patients that are non-carriers for any CYP2C19 variants (p=0.0608) (125). Since ticagrelor is a substrate for platelet GP (126), the impact of ABCB1 polymorphisms has been investigated in both trials mentioned above without any significant effects. Finally, polymorphic variants in the target genes P2RY12, P2RY1 and ITGB3 did not influence inhibition of ADP-induced aggregation by ticagrelor (127).

**Oral thrombin inhibitors**

There is still a lack of published systematic pharmacogenomic data for the newly approved oral thrombin inhibitors dabigatran, rivaroxaban and apixaban. CYP450 enzymes or other oxidoreductase are not involved in the conversion of the prodrug dabigatran etexilate into the active drug dabigatran. Dabigatran is mainly excreted unchanged. Although a considerable number of metabolites have been identified, they account only for up to 0.6% of the dose in urine (128). One important metabolic step, however, is the bioactivation of dabigatran etexilate via esterase-catalysed hydrolysis into dabigatran, the only compound which is detectable in human plasma. It has been suggested that the hydrolysis of dabigatran etexilate may be mediated by microsomal CES (128) with potential impact of genetic variation in CES genes (129).

**Thrombin receptor (PAR1) antagonists**

Only few polymorphism of the PAR-1 receptor gene have been reported in clinical studies. The intervening sequence IVS-14 A/T intronic variation (rs168753) has been described to correlate with reduced PAR-1 receptors on the platelet surface and with the response to agonist stimulation (130). Additionally, the insertion/deletion variant 506 I/D (rs11267092) has been evaluated for its protective role in venous thromboembolism (131). None of these variants has been investigated for its association with pharmacodynamic or clinical efficacy of oral PAR1-antagonists vorapaxar (SCH 530348) and edoxaban (E5555).

**Role of genetic testing to individualise antiplatelet therapy**

Point-of-care genetic testing has been considered to promote clinical utility of CYP2C19 testing in clinical practice. Two systems have been currently marketed: the Spartan RX CYP2C19 (Spartan Bioscience Inc) and the Verigen system (Nanosphere Inc). Two randomised phase II clinical trials have been recently published to evaluate the usefulness of these systems in monitoring antiplatelet therapy according to CYP2C19 loss-of-function allelic. ELEVATE-TIMI 56 was a multicentre, randomised, double-blind trial that enrolled and genotyped 333 patients with stable cardiovascular disease. Patients were tested for CYP2C19*2 status by Verigen engine. Maintenance doses of clopidogrel for four treatment periods, each lasting approximately 14 days, was adjusted according to genotype. Two hundred forty-seven non-carriers for CYP2C19*2 received 75 and 150 mg clopidogrel daily (two periods each), whereas 86 carriers (80 heterozygotes, 6 homozygous variant patients) were treated with increasing doses up to 300 mg daily. Platelet function was monitored at (vasodilator-stimulated phosphoprotein [VASP] phosphorylation and VerifyNow P2Y12 assays) as well as adverse drug reactions. CYP2C19*2 heterozygotes had sig-
significantly higher on-treatment platelet reactivity with clopidogrel 75 mg than did non-carriers. Among CYP2C19*2 heterozygotes, doses up to 300 mg daily significantly reduced platelet reactivity measured by VASP (PRI) and VerifyNow (PRU) indicating a dose relationship of platelet aggregation inhibition in carriers of one CYP2C19 allele. In contrast, dose increase did not show any significant improvement of platelet reactivity in homozygous variant patients (132).

In the Reassessment of Antiplatelet Therapy Using an Individualised Strategy Based on Genetic Evaluation (RAPID GENE) study, genotype adjusted antiplatelet therapy in patients with symptomatic CAD was tested as proof-of-principle. Two hundred patients undergoing PCI for ACS or stable angina were randomised to prasugrel 10 mg/day in case of CYP2C19*2 carrier status, clopidogrel 75 mg/day for those non-carrying the CYP2C19*2 allele at point-of-care genotyping, or standard antiplatelet therapy with clopidogrel 75 mg/day without prospective genotyping. Platelet-function testing was performed one week later, and those randomised to standard care were retrospectively genotyped. Sensitivity of the genotyping assay for the allele, compared with gold-standard DNA sequencing, was 100%, with a specificity of 99.4%.

A total 187 patients completed the follow-up (91 of point-of-care genotyping group vs 96 of the standard treatment). None of the 23 patients carrying at least one CYP2C19*2 allele in the rapid genotyping group had a P2Y12 reactivity unit value higher than 234 at day 7 after dual antiplatelet treatment, which is a marker associated with increased adverse cardiovascular events compared with seven given standard treatment (p=0.0092) (133). This study supports the assumption that point-of-care genetic testing for CYP2C19 after PCI can help to identify patients at risk in clopidogrel non-response, although the isolated LOF-genotype only explains up to 12% of response variability (3).

Conclusions

Although some conflicting data with regard to the prognostic role of genetic polymorphisms affecting platelet function and efficacy of antiplatelet drug therapy exist, there is a growing body of evidence from genome-wide association and functional genomic studies that variability of platelet activation largely depends on genetic inheritance. Involvement of additional technologies like metabolomics through multi-genetic associations and determination of the adequate platelet phenotype to monitor antiplatelet drug effects may be crucial to develop sensitive models for risk assessment in cardiovascular patients in the future. Large-scale pharmacogenomic cohort studies are required with strictly defined platelet function/disease/treatment phenotypes. To this end, the euphoria to determine individual risk and effects of antiplatelet therapies by means of genetic testing only should not be overestimated, as non-genetic risk factors including disease-related factors additionally play an important role. However, the development and availability of novel antiplatelet strategies such as prasugrel, ticagrelor and PAR1-antagonists will hopefully allow us to tailor antiplatelet therapies more precisely also including genetic and non-genetic risk profiles on an individual basis to find the safest and most efficacious treatment option in future.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (Grant SCHW 858/1-1 and Klinische Forschergruppe 274 Platelets – Molecular Mechanisms and Translational Implications), the FP7 EU Initial Training Network program Fighting-DrugFailure (Grant PITN-GA-2009-238132), and the Robert Bosch Stiftung, Germany.

Conflicts of interest

None declared.

References


Thrombosis and Haemostasis 110.5/2013 © Schattauer 2013

Downloaded from www.thrombosis-online.com on 2017-07-06, IP: 54.191.40.80
For personal or educational use only. No other uses without permission. All rights reserved.
Platelets: basic mechanisms and translational implications


Hulot JS, Collet JP, Cayla G, et al. CYP2C19 but not PON1 genetic variants in- 
cluencing clopidogrel pharmacokinetics, pharmacodynamics, and clinical effi- 
cacy in post-myocardial infarction patients. Circ Cardiovasc Interv 2011; 4: 
422–428.

on clopidogrel efficacy and cardiovascular events in the Clopidogrel in the Un- 
stable Angina to Prevent Recurrent Events trial and the Atrial Fibrillation 
Clopidogrel Trial with Irbesartan for Prevention of Vascular Events. Circ Car- 
diovasc Genet 2012; 5: 250–256.

on clinical outcomes in patients treated with clopidogrel after an acute myo- 


110. Trenk D, Hochholzer W, Fromm MF, et al. Paraoxonase-1 Q192R polymor- 
phism and antiplatelet effects of clopidogrel in patients undergoing elective 

of the paraoxonase-1 Q192R genetic variant on clopidogrel responsiveness and 


113. Dansette PM, Rosi J, Bertho G, et al. Cytochromes P450 catalyze both steps of 
the major pathway of clopidogrel bioactivation, whereas paraoxonase catalyzes 
the formation of a minor thiol metabolite isomer. Chem Res Toxicol 2012; 25: 
348–356.

tative determination of the clopidogrel active metabolite isomers in human 

115. Tarkiainen EK, Backman JT, Neuvonen M, et al. Carboxylesterase 1 polymor- 

prasugrel, a thienopyridine antiplatelet agent, with the cytochromes P450. 
Drug Metab Dispos 2006; 34: 600–607.

following maintenance doses of prasugrel and clopidogrel in Chinese carriers of 

on-clopidogrel platelet reactivity post-stenting more effectively than high-dose 
(150-ng) clopidogrel: the importance of CYP2C19*2 genotyping. JACC Car- 

119. Cuisset T, Loosveld M, Morange PE, et al. CYP2C19*2 and *17 alleles have a 
significant impact on platelet response and bleeding risk in patients treated 
with prasugrel after acute coronary syndrome. JACC Cardiovasc Interv 2012; 5: 
1280–1287.

120. Grosdidier C, Quilici J, Loosveld M, et al. Effect of CYP2C19*2 and *17 Gen- 
etic Variants on Platelet Response to Clopidogrel and Prasugrel Maintenance 
Dose and Relation to Bleeding Complications. Am J Cardiol 2013; Epub ahead of 
print.

and cardiovascular outcomes after treatment with clopidogrel and prasugrel in 
the TRITON-TIMI 38 trial: a pharmacogenetic analysis. Lancet 2010; 376: 
1312–1319.

122. Zhou D, Andersson TB, Grimm SW. In vitro evaluation of potential drug-drug 
interactions with ticagrelor: cytochrome P450 reaction phenotyping, in- 
hibition, induction, and differential kinetics. Drug Metab Dispos 2011; 39: 
703–710.

CYP2C19 genotype and pharmacodynamics in patients treated with ticagrelor 
versus clopidogrel: the ONSET/OFFSET and RESPOND genotype studies. Circ 

and ABCB1 single nucleotide polymorphisms on outcomes of treatment with 
ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy 

125. Teng R. Pharmacokinetic, pharmacodynamic and pharmacogenetic profile of 

126. Storey RF, Thornton MS, Lawrance R, et al. Ticagrelor yields consistent dose- 
dependent inhibition of ADP-induced platelet aggregation in patients with 
atherosclerotic disease regardless of genotypic variations in P2RY12, P2RY1, 

of the oral direct thrombin inhibitor, dabigatran, in humans. Drug Metab Dis- 

128. Paré G, Eriksson N, Lehr T, et al. RE-LY-Genetics: Genetic determinants of 
dabigatran plasma levels and their relation to clinical response. European So- 
ciety of Cardiology 2012 Congress; August 29, 2012, Munich, Germany.

PAR-1 gene is associated with platelet receptor density and the response to 

(protease-activated receptor 1) gene polymorphism toward venous throm- 

CYP2C19 genotype and the effect on platelet reactivity in patients with stable 

132. Roberts JD, Wells GA, Le May MR, et al. Point-of-care genetic testing for per- 
sonalisation of antiplatelet treatment (RAPID GENE): a prospective, random- 