CYP2C19 Polymorphism and Clopidogrel: Testing, Testing, *1*2*3... 

Objectives

1. Explain the basic concepts of pharmacogenetics and their relationship to drug activity
2. Describe the influence of CYP2C19 polymorphism on the activation and antiplatelet effects of clopidogrel
3. Evaluate the relationship between CYP2C19 reduced-function alleles and clinical outcomes

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Central Texas Veterans Health Care System
Background

I. Clopidogrel

a. **Black box warning**

```
WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS

The effectiveness of Plavix is dependent on its activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19 [see Warnings and Precautions (5.1)]. Plavix at recommended doses forms less of that metabolite and has a smaller effect on platelet function in patients who are CYP2C19 poor metabolizers. Poor metabolizers with acute coronary syndrome or undergoing percutaneous coronary intervention treated with Plavix at recommended doses exhibit higher cardiovascular event rates than do patients with normal CYP2C19 function. Tests are available to identify a patient's CYP2C19 genotype; these tests can be used as an aid in determining therapeutic strategy [see Clinical Pharmacology (12.3)]. Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers [see Dosage and Administration (2.3)].
```

b. **Activation and mechanism**

i. Clopidogrel is a pro-drug that requires hepatic bioactivation

1. 85% of the dose is hydrolyzed by ubiquitous esterases, which leaves only 15% to be converted to the active form²

2. The activation of clopidogrel is a two step process.³
   (a) Clopidogrel is converted to 2-oxoclopidogrel via CYP2C19, CYP1A2, and CYP2B6 with each enzyme contributing 45%, 36%, and 19%, respectively
   (b) 2-oxoclopidogrel is converted to the thiol active metabolite via CYP3A4/5, CYP2B6, CYP2C19, and CYP2C9 with a contribution reported to be 40%, 33%, 21%, and 7%, respectively

3. The thiol active metabolite irreversibly forms a disulfide bridge with a cysteine residue within the P2Y12 receptor⁴
   (a) This action prevents activation of the GPIIb/IIIa receptor complex, thereby inhibiting aggregation for the platelet’s lifespan (about 10 days)

![Clopidogrel Activation Diagram](image)

Figure 1. Sangkuhl K, et al. Pharmacogenet Genomics 2010;20:463-465
c. **Indications**
   i. Recent myocardial infarction, stroke, or established peripheral artery disease
      1. AHA/ASA
         (a) Prior stroke or TIA – 75 mg daily as initial monotherapy (Class IIa recommendation, Level B evidence; aspirin is Class I, Level A)
      2. ACCF/AHA
         (a) PAD – 75 mg daily as an alternative to aspirin (Class I, Level B; aspirin is Class I, Level B)
   ii. Unstable angina/non-ST-elevation myocardial infarction

<table>
<thead>
<tr>
<th>ACCF/AHA guidelines</th>
<th>Loading dose</th>
<th>Maintenance dose</th>
<th>Length of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCI</td>
<td>300-600 mg</td>
<td>75 mg daily</td>
<td>1 month (ideally 1 year)</td>
</tr>
<tr>
<td>Bare-metal or drug-eluting stent</td>
<td>75 mg daily</td>
<td>At least 12 months</td>
<td></td>
</tr>
</tbody>
</table>

iii. ST-elevation myocardial infarction

<table>
<thead>
<tr>
<th>ACC/AHA guidelines</th>
<th>Loading dose</th>
<th>Maintenance dose</th>
<th>Length of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinolysis or no reperfusion therapy</td>
<td>300 mg (age &lt;75) reasonable</td>
<td>75 mg daily</td>
<td>At least 14 days (1 year reasonable)</td>
</tr>
<tr>
<td>PCI† Bare-metal or drug-eluting stent</td>
<td>300-600 mg</td>
<td>75 mg daily</td>
<td>At least 12 months</td>
</tr>
<tr>
<td>Non-primary PCI‡ +/- fibrinolysis</td>
<td>300-600 mg</td>
<td>75 mg daily</td>
<td>At least 12 months</td>
</tr>
</tbody>
</table>

d. **Effect size**
   i. PCI with stenting – 75-85% risk reduction of cardiovascular death, myocardial infarction, or stroke
   ii. Conservative management – 20% risk reduction of cardiovascular death, myocardial infarction, or stroke
   iii. Atrial fibrillation (unlabeled indication) – 10% risk reduction of cardiovascular death, myocardial infarction, stroke, or systemic embolism

e. **Response variability**
   i. Clopidogrel is known to have high interindividual variability with decreased response being described as non-responsiveness or resistance

Figure 2. Interindividual variability in platelet response to clopidogrel after stenting. Clopidogrel “resistance” was defined as an absolute change in platelet aggregation <10% before and after clopidogrel administration in response to 5μmol/L ADP.

Figure 3. Relationship between IPA by clopidogrel 300 mg or prasugrel 60 mg in response to 20 Amol/L ADP 24 hours after the LD. Subjects were administered both clopidogrel and prasugrel in a crossover fashion.16


ii. Clopidogrel non-responsiveness and outcomes
   1. Sofi et al, 2010 – meta-analysis17
      (a) 4564 patients with stable angina, chronic CAD, or ACS
      (b) Endpoint – major adverse cardiac events including death from cardiovascular causes, myocardial infarction, stroke, unstable angina, ischemic recurrences, or stent thrombosis
      (c) Results:
         (i) Clopidogrel non-responsiveness associated with an increased risk of recurrent cardiovascular events
         (ii) OR 3.58; 95% CI, 2.54-5.05 (P < 0.00001) – after adjustment for heterogeneity

iii. Proposed causes of response variability18

- Cellular factors:
  - Increased platelet turnover
  - Decreased metabolic activity
  - Up-regulation of P2Y12 or P2Y1
  - Up-regulation of P2Y-independent pathways

- Genetic Factors:
  - CYP polymorphisms
  - GPIa polymorphisms
  - PY212 polymorphisms
  - GPIIIa polymorphisms

- Clinical factors:
  - Age
  - Compliance
  - Under-dosing
  - Poor absorption
  - Drug interactions
  - ACS
  - Diabetes
  - BMI

Reduced response to clopidogrel
II. Pharmacogenetics

a. Definition – the search for genetic variations that lead to interindividual differences in drug response
b. Applications:
   i. Predict responders
      1. Trastuzumab
   ii. Prevent adverse reactions
      1. Abacavir
   iii. Choose appropriate doses
      1. 6-mercaptopurine
   iv. Drug development
      1. Avoidance of molecules metabolized by polymorphic enzymes
c. Genetic differences
   i. Central dogma theory of molecular biology
      1. DNA is transcribed into RNA which is translated into a protein
      2. Three nucleotides form a codon
      3. A series of codons constitutes a gene
         (a) Genes encode proteins which may affect drug response:
            (i) Metabolizing enzyme
            (ii) Transporter
            (iii) Receptor
   ii. Human DNA sequence
      1. 99.9% identical from person to person
      2. 3 billion total nucleotides (0.1% difference is larger than it seems)
         (a) Differences can predict pharmacokinetic and pharmacodynamic response to drugs
   iii. Examples of gene mutations (source of genetic differences):
      1. Single nucleotide polymorphism – one nucleotide base pair replaces another
      2. Insertion/deletions – nucleotide or nucleotide sequence is added or deleted
      3. Tandem repeats – nucleotide sequence repeats in tandem (e.g. AGAGAGAG)
      4. Frameshift mutation – an insertion/deletion mutation in which the change in number of nucleotides is not a multiple of three
      5. Defective splicing – internal polypeptide segment is abnormally removed and remaining ends are joined
      6. Premature stop codon – premature termination of the polypeptide chain
      7. Copy number variations – an abnormal number of copies of a gene
   iv. Polymorphisms – variation (mutation) in at least 1% of population
      1. Examples:
         (a) Eye color
         (b) Hair color
         (c) Blood type
         (d) Drug metabolizing enzymes
d. **Pharmacogenetics and CYP enzymes**
   i. Over 50 cytochrome P450 isoenzymes
      1. Three families – CYP1, CYP2, CYP3
      2. Fifteen known to metabolize drugs
      3. At least seven with documented polymorphisms – CYP2A6, 2C9, 2C19, 2D6, 3A4/5, 1A2
   ii. Two copies of each gene that encode for a CYP enzyme
      1. Each copy is referred to as an allele
   iii. Example of a polymorphic CYP enzyme

<table>
<thead>
<tr>
<th>Allelic genotype</th>
<th>Metabolizer phenotype</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>More than 2 functional alleles or alleles with increased activity</td>
<td>Ultrarapid</td>
<td>High metabolic capacity</td>
</tr>
<tr>
<td>2 normal (wild type) alleles</td>
<td>Extensive</td>
<td>Normal capacity</td>
</tr>
<tr>
<td>1 functional and 1 defective allele or 2 partially defective alleles</td>
<td>Intermediate</td>
<td>Reduced capacity</td>
</tr>
<tr>
<td>2 defective alleles</td>
<td>Poor</td>
<td>No functional enzyme</td>
</tr>
</tbody>
</table>

### III. Clopidogrel pharmacogenetics and CYP2C19

a. **CYP2C19 polymorphism**
   i. Alleles, genotype, and phenotype\(^{2,20}\)

<table>
<thead>
<tr>
<th>CYP2C19 allele</th>
<th>Functional status</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>Functional/normal activity/wild type</td>
</tr>
<tr>
<td>*17</td>
<td>Increased function/increased activity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Metabolizer phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>*17/*17</td>
<td>Ultrarapid</td>
</tr>
<tr>
<td>*1/*17</td>
<td>Ultrarapid</td>
</tr>
<tr>
<td>*1/*1</td>
<td>Extensive</td>
</tr>
<tr>
<td>*1/*2-8</td>
<td>Intermediate†</td>
</tr>
<tr>
<td>*17/*2-8</td>
<td>Intermediate†</td>
</tr>
<tr>
<td>*2-8/*2-8</td>
<td>Poor</td>
</tr>
</tbody>
</table>

\(^{†}\)Phenotype not well determined

ii. **Frequencies of CYP2C19 alleles\(^2\)**

<table>
<thead>
<tr>
<th>Allele</th>
<th>African</th>
<th>American</th>
<th>European</th>
<th>East Asian</th>
<th>South/ Central Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>68%</td>
<td>69%</td>
<td>63%</td>
<td>60%</td>
<td>62%</td>
</tr>
<tr>
<td>*2</td>
<td>15%</td>
<td>12%</td>
<td>15%</td>
<td>29%</td>
<td>35%</td>
</tr>
<tr>
<td>*3</td>
<td>0.5%</td>
<td>0.03%</td>
<td>0.4%</td>
<td>8.9%</td>
<td>2.4%</td>
</tr>
<tr>
<td>*4</td>
<td>0.09%</td>
<td>0.24%</td>
<td>0.25%</td>
<td>0.05%</td>
<td>0.00%</td>
</tr>
<tr>
<td>*5</td>
<td>ND</td>
<td>0.00%</td>
<td>0.007%</td>
<td>0.06%</td>
<td>0.00%</td>
</tr>
<tr>
<td>*6</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.017%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>*7</td>
<td>ND</td>
<td>ND</td>
<td>0.00%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>*8</td>
<td>0.00%</td>
<td>0.12%</td>
<td>0.35%</td>
<td>0.00%</td>
<td>ND</td>
</tr>
<tr>
<td>*17</td>
<td>16%</td>
<td>18%</td>
<td>21%</td>
<td>2.7%</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND – not determined

b. **Why is the focus on CYP2C19?**
   i. Pharmacokinetic and pharmacodynamic effects of reduced-function alleles
      1. Mega et al, 2009\(^{21}\)
         (a) 162 healthy subjects were included from six studies involving clopidogrel treatment
         (b) Polymorphic CYP enzymes tested – 2C19, 2C9, 2B6, 3A5, 1A2
(c) Endpoints:
   (i) PK – AUC$_{0-t}$ of active metabolite for carriers of at least one reduced-function CYP allele compared to noncarriers (AUC$_{0-t}$ computed by noncompartmental methods with the use of the log-linear trapezoidal method)
   
   (ii) PD – Absolute difference in reduction of maximal platelet aggregation ($\Delta$MPA) in response to clopidogrel between carriers and noncarriers ($\Delta$MPA assessed by the use of light transmission aggregometry in response to 20 µM of ADP)

(d) Results:
   (i) Carriers of at least one CYP2C19 reduced-function allele had a relative reduction of 32.4% in plasma exposure to the active metabolite as compared to noncarriers ($P < 0.001$)
   (ii) Carriers of at least one CYP2C19 reduced-function allele had an absolute $\Delta$MPA in response to clopidogrel that was 9% less than noncarriers ($P < 0.001$), relative risk reduction of 25%
   (iii) Carriers of other CYP reduced-function alleles did not have significant differences in PK and PD endpoints compared to noncarriers

Figure 4. Model-based estimates show the effects associated with carriage of at least one reduced-function allele in five genes encoding cytochrome P-450 enzymes on the pharmacokinetic and pharmacodynamic responses to clopidogrel. The threshold for statistical significance was $P < 0.01$. The horizontal lines represent 95% confidence intervals.


(c) Response variability due to CYP2C19
   i. Genome wide association study (GWAS)
      1. Shuldiner et al, 2009
   
      (a) 429 relatively healthy Amish subjects received clopidogrel for 7 days
      (b) Endpoint – ADP stimulated platelet aggregation
      (c) Results:
         (i) Patient response to clopidogrel was highly heritable
         (ii) Age, BMI, and lipid levels predicted <10% of variation in platelet aggregation
         (iii) CYP2C19*2 allele predicted 12% of variation
Literature review

IV. Supporting evidence

a. Cytochrome P-450 polymorphisms and response to clopidogrel

i. Objective

1. To examine the association between genetic variants in CYP genes and cardiovascular outcomes in a separate cohort of 1477 subjects with acute coronary syndromes who were treated with clopidogrel

ii. Methods

1. Design

(a) Genetic substudy of the treatment arm of a phase 3, randomized, double-blind, parallel-group, multinational, clinical trial (TRITON-TIMI 38 trial)

(b) Analysis of clopidogrel arm only, “treatment only” design

2. Subjects

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria (major)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General:</td>
<td>General:</td>
</tr>
<tr>
<td>• Received clopidogrel for acute coronary syndrome with scheduled percutaneous coronary intervention (PCI)</td>
<td>• Pregnancy</td>
</tr>
<tr>
<td>• Provided a DNA sample</td>
<td>• Age &lt; 18 years</td>
</tr>
<tr>
<td>UA or NSTEMI:</td>
<td>Increased risk of bleeding:</td>
</tr>
<tr>
<td>• Ischemic symptoms lasting 10 min or more occurring within 72 hrs before randomization</td>
<td>• Anemia (Hgb &lt; 10 g/dL)</td>
</tr>
<tr>
<td>• TIMI risk score of 3 or more</td>
<td>• Thrombocytopenia (Plt &lt; 100 K/mm$^3$)</td>
</tr>
<tr>
<td>• Either ST-segment deviation of 1 mm or more or elevated levels of cardiac biomarkers</td>
<td>• History of pathological intracranial findings</td>
</tr>
<tr>
<td>STEMI:</td>
<td>• Use of any thienopyridine within 5 days before enrollment</td>
</tr>
<tr>
<td>• Within 12 hrs after the onset of symptoms if primary PCI was planned or within 14 days after receiving medical treatment for STEMI</td>
<td>• Fibrinolytic use (within 24 hrs for fibrin specific and 48 hrs for nonfibrin specific)</td>
</tr>
<tr>
<td>Cardiovascular:</td>
<td>Cardiovascular:</td>
</tr>
<tr>
<td>• Cardiogenic shock</td>
<td>• Refractory ventricular arrhythmias</td>
</tr>
<tr>
<td>• New York Heart Association class IV CHF</td>
<td></td>
</tr>
</tbody>
</table>

3. Treatment

(a) Clopidogrel 300 mg loading dose, followed by a 75 mg daily maintenance dose for up to 15 months

4. Comparison groups-CYP2C19 genes

<table>
<thead>
<tr>
<th>Dichotomous classification</th>
<th>Associated genotypes</th>
<th>Associated phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncarrier of a reduced-function allele</td>
<td>*1/*1, *1/*17 *17/*17</td>
<td>Extensive metabolizer Ultrarapid metabolizer</td>
</tr>
<tr>
<td>Carrier of a reduced-function allele</td>
<td>*1/*2-5, *1/*8 *2/*2-5, *2/*8</td>
<td>Intermediate metabolizer Poor metabolizer</td>
</tr>
</tbody>
</table>

5. Primary endpoint

(a) Composite death from cardiovascular causes, myocardial infarction, or stroke

6. Secondary endpoint

(a) Definite or probable stent thrombosis

7. Safety outcome

(a) TIMI major or minor bleeding not related to coronary-artery bypass graft

8. Note

(a) Outcomes were adjudicated by a clinical events committee whose members were unaware of study-group assignments
9. Statistical analysis
   (a) Rates of outcomes were expressed as Kaplan-Meier estimates at 15 months
   (b) The Gehan-Wilcoxon test was used for the primary endpoint
   (c) The Log-rank test was used for other outcomes
   (d) Hazard ratios and 95% confidence intervals were calculated on the basis of Cox-proportional-hazards models
   (e) Two-sided P values of less than 0.05 were considered significant

iii. Results
1. Baseline characteristics
   (a) DNA samples were available for 1477 subjects who were assigned to clopidogrel in the TRITON-TIMI 38 trial
   (b) Mean age = 60.1 +/- 11.1 years
   (c) 29.3% were female, 70.7% male
   (d) 97.6% Caucasian
   (e) 71% with NSTEMI or UA, 29% STEMI
   (f) 100% received PCI, 47% received drug-eluting stent
   (g) 27.1% had at least one reduced-function CYP2C19 allele

2. Primary endpoint
   (a) 395 subjects carrying at least one CYP2C19 reduced-function allele were at higher risk for the primary endpoint
      (i) 12.1% vs. 8.0%; HR for carriers 1.53; 95% CI, 1.07 to 2.19 (P = 0.01)
      (ii) The components of the primary outcome were directionally consistent with the results of the composite endpoint

3. Secondary and safety endpoints
   (a) The risk of stent thrombosis in carriers of a CYP2C19 reduced-function allele was three times that among noncarriers
      (i) 2.6% vs. 0.8%; HR 3.09; 95% CI, 1.19 to 8.00 (P = 0.02)
   (b) There were no differences between groups for the safety outcome of major or minor bleeding

iv. Author’s conclusion
   1. “Carriers of a reduced-function CYP2C19 allele have significantly lower levels of the active metabolite of clopidogrel, diminished platelet inhibition, and a higher rate of major adverse cardiovascular events, including stent thrombosis.”

v. Critique

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Mechanistically plausible</td>
<td>- Retrospective analysis</td>
</tr>
<tr>
<td>- Included genotyping for many CYP2C19 genetic variants (not just the *1, *2, *3, and *17 alleles)</td>
<td>- 97.6% white population</td>
</tr>
<tr>
<td>- Appropriate endpoints</td>
<td>- Baselines characteristics reported but no mention of whether there were significant differences between groups</td>
</tr>
<tr>
<td>- Components of the primary endpoint were directionally consistent with the overall results</td>
<td>- Compliance?</td>
</tr>
<tr>
<td></td>
<td>- Design does not control for potential effects of these genes via some other mechanism</td>
</tr>
<tr>
<td></td>
<td>- Too few homozygotes to analyze separately</td>
</tr>
</tbody>
</table>

b. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis
   i. Methods
      1. Study selection (includes TRITON-TIMI 38 substudy)

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic studies involving clopidogrel and CYP2C19</td>
<td>No results for specific number of variant alleles, only for carrier state status</td>
</tr>
<tr>
<td>Majority of patients were invasively managed (PCI)</td>
<td>Clinical outcomes not measured</td>
</tr>
<tr>
<td>Treatment followed current guidelines</td>
<td>Not a cohort study or clinical trial</td>
</tr>
</tbody>
</table>

2. Comparison groups
   (a) Patients classified as carrying zero, one, or two CYP2C19 reduced-function alleles

3. Endpoints
   (a) Composite death from cardiovascular causes, MI, or stroke
   (b) Stent thrombosis

ii. Results
   1. Baseline characteristics
      (a) 9685 patients from nine studies contributed to the composite outcome
      (b) 54.5% had ACS
      (c) 91.3% received PCI
      (d) 26.3% had one and 2.2% had two reduced-function alleles
2. Endpoints

<table>
<thead>
<tr>
<th>CYP2C19 reduced-function alleles</th>
<th>HR (95% CI)†</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composite endpoint</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>1.55 (1.11-2.27)</td>
<td>0.01</td>
</tr>
<tr>
<td>Two</td>
<td>1.76 (1.24-2.50)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Stent thrombosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>2.67 (1.69-4.22)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Two</td>
<td>3.97 (1.75-9.02)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

†As compared to zero reduced-function alleles

iii. Author’s conclusion
1. “Among patients treated with clopidogrel for PCI, carriage of even one reduced-function CYP2C19 allele appears to be associated with a significantly increased risk of major adverse cardiovascular events, particularly stent thrombosis.”

c. Supporting evidence implications
i. For patients treated with clopidogrel and planned PCI, having at least one reduced-function may increase risk for cardiovascular events by about 50%
ii. Reduced-function alleles may increase risk for stent thrombosis by nearly a factor of three
iii. Results cannot be applied to:
1. Conservatively managed patients
2. Patients treated for atrial fibrillation

V. Refuting evidence
a. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment
i. Objective
1. To examine if the benefits of clopidogrel vs. placebo, in patients treated for acute coronary syndromes, differ between various categories of CYP2C19 metabolizer phenotype

ii. Methods
1. Design
   (a) Genetic substudy of a randomized, double-blind, placebo-controlled trial (CURE trial)
   (b) Included both clopidogrel and placebo groups in the genetic analysis, “effect modification” design
2. Subjects

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria (major)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 18 years</td>
<td>STEMI</td>
</tr>
<tr>
<td><strong>Acute coronary syndromes (not STEMI)</strong></td>
<td>Contraindications to antithrombotic/antiplatelet therapy</td>
</tr>
<tr>
<td>Hospitalized within 24 hrs of symptom onset</td>
<td>High bleeding risk</td>
</tr>
<tr>
<td>EKG changes or elevation of serum cardiac enzymes/markers</td>
<td>Severe heart failure</td>
</tr>
<tr>
<td>Consented to genetic study</td>
<td>Taking oral anticoagulants</td>
</tr>
<tr>
<td></td>
<td>Received GPIIb/IIIa inhibitors within last 3 days</td>
</tr>
<tr>
<td></td>
<td>Coronary revascularization within last 3 months</td>
</tr>
<tr>
<td></td>
<td>Ethnicities other than European or Latin American ancestry</td>
</tr>
</tbody>
</table>

3. Treatment
   (a) Clopidogrel 300 mg loading dose, followed by 75 mg daily or placebo for up to 12 months
4. Comparison groups-CYP2C19 genes

<table>
<thead>
<tr>
<th>Metabolizer phenotype</th>
<th>Associated genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>*2/*2, *2/*3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>*1/*2, *1/*3</td>
</tr>
<tr>
<td>Extensive</td>
<td>*1/*1</td>
</tr>
<tr>
<td>Ultrarapid</td>
<td>*1/*17, *17/*17</td>
</tr>
<tr>
<td>Unknown</td>
<td>*2/*17, *3/*17</td>
</tr>
</tbody>
</table>

5. First primary endpoint
   (a) Composite death from cardiovascular causes, myocardial infarction, or stroke

6. Second primary outcome
   (a) Composite of first primary outcome plus recurrent ischemia or hospitalization from unstable angina

7. Safety outcome
   (a) Major bleeding (requiring 2 or more units of blood)

8. Statistical analysis
   (a) Effect of clopidogrel as compared with placebo according to CYP2C19 metabolizer phenotype was assessed with Cox proportional-hazards regression
   (b) Results presented from a model adjusting for age, sex, and ancestry
   (c) Treatment effect according to loss-of-function and gain-of-function carrier status was examined with the use of analogous models
   (d) Two-sided P values of less than 0.05 were considered significant

9. Power analysis (not prospectively calculated)
   (a) A hazard ratio of 1.53 was the smallest increase in cardiovascular risk for loss-of-function carriers reported in the literature at the time of the study
      (i) Using this HR, this study had more than 85% power to detect an interaction of treatment with carrier status with respect to the primary efficacy outcome and 95% power to detect an interaction in the second primary composite outcome

iii. Results

1. Baseline characteristics
   (a) 5059 patients were genotyped, with 2549 in the clopidogrel group and 2510 in the placebo group
   (b) Mean age = 63.8 +/- 11.0 years
   (c) 40.9% were female, 59.1% male
   (d) 85.9% were of European ancestry, 14.1% Latin American
   (e) Only 18% received PCI, 14.5% received a stent (0% drug-eluting stent)
   (f) 2.3% poor, 17.5% intermediate, 33.4% ultrarapid, and 40.3% extensive metabolizers

2. First and second primary outcome
   (a) The effects of clopidogrel were not significantly different between subgroups defined according to metabolizer status for both the first and second primary composite efficacy outcomes (P = 0.12 and P = 0.29 for heterogeneity, respectively)

3. Safety outcome
   (a) Major bleeding with clopidogrel as compared to placebo was not significantly different between subgroups defined according to metabolizer status (P = 0.64 for heterogeneity)

4. Other analyses
   (a) The effect of clopidogrel as compared with placebo in reducing the first primary outcome was similar between carriers of loss-of-function alleles and noncarriers
      (i) HR for carriers 0.69; 95% CI, 0.49 to 0.98; HR for noncarriers 0.72; 95% CI, 0.59 to 0.87 (P = 0.84 for the interaction)
   (b) A significant interaction was observed between gain-of-function allele carrier status and noncarriers with respect to the first primary outcome, such that carriers had a more pronounced reduction in cardiovascular events with clopidogrel treatment as compared with placebo than did noncarriers
      (i) HR for carriers 0.55; 95% CI, 0.42 to 0.73; HR for noncarriers 0.85; 95% CI, 0.68 to 1.05 (P = 0.02 for the interaction)

iv. Author's conclusion
   1. “CYP2C19 loss-of-function variants do not modify the efficacy and safety of clopidogrel. Therefore, loss-of-function allele carrier status should not preclude the use of clopidogrel at currently recommended doses in patients with acute coronary syndromes whose condition is being managed conservatively.”
v. Critique

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Effect modification design controls for sources of confounding, such as possible pleiotropic genetic effects</em></td>
<td><em>Retrospective analysis</em></td>
</tr>
<tr>
<td>Large number of subjects</td>
<td><em>Excluded STEMI patients</em></td>
</tr>
<tr>
<td>The benefits of clopidogrel were seen in all genetic cohorts</td>
<td>Only analyzed European and Latin American ancestry</td>
</tr>
<tr>
<td>Appropriate endpoints</td>
<td>Baselines characteristics reported but no mention of whether there were significant differences between groups</td>
</tr>
<tr>
<td><strong>Overwhelming majority of subjects were conservatively managed, in which the affects of clopidogrel are reduced compared to the invasive approach</strong></td>
<td>*Did not genotype for all CYP2C19 reduced function alleles (only *2 and <em>3)</em></td>
</tr>
<tr>
<td><strong>Compliance?</strong></td>
<td></td>
</tr>
<tr>
<td>Differences is stent thrombosis not analyzed</td>
<td></td>
</tr>
<tr>
<td>Drug-eluting stents were not in use at the time of the CURE trial</td>
<td></td>
</tr>
</tbody>
</table>

b. *Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial*

i. **Objective**
   1. To examine if the benefits of ticagrelor vs. clopidogrel, in patients treated for acute coronary syndromes, differ between CYP2C19 reduced-function allele carriers and noncarriers

ii. **Methods**
   1. **Design**
      (a) Genetic substudy of a prospective, randomized, double-blind, double-dummy, international, multicenter phase 3 trial (PLATO trial)
      (b) Included both clopidogrel and ticagrelor groups in the genetic analysis, “effect modification” design
      (c) Ticagrelor needs no activation step to bind to the P2Y12 receptor, so it should not be affected by CYP2C19 function and can serve as the control group

   2. **Subjects**

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria (major)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS symptoms within 24 hrs; consented to genetic study</td>
<td>Any contraindication for the use of clopidogrel</td>
</tr>
<tr>
<td>ACS without ST elevation must have at least 2 of the following 3:</td>
<td>Fibrinolytic therapy within 24 hrs</td>
</tr>
<tr>
<td>• ST changes indicating ischemia</td>
<td></td>
</tr>
<tr>
<td>• Positive biomarkers</td>
<td>Need for oral anticoagulation</td>
</tr>
<tr>
<td>• Age ≥ 60, previous MI or CABG, CAD, previous CVA or TIA, carotid stenosis, cerebral revascularization, diabetes, PAD, CKD stage III or worse</td>
<td></td>
</tr>
<tr>
<td>STEMI must have the following 2 conditions:</td>
<td></td>
</tr>
<tr>
<td>• Persistent ST elevation in 2 contiguous leads or a new left bundle-branch block</td>
<td>Increased risk for bradycardia</td>
</tr>
<tr>
<td>• Intention to perform primary PCI</td>
<td>Concomitant therapy with strong CYP3A4 inhibitor or inducer</td>
</tr>
</tbody>
</table>
3. Treatment
   (a) Clopidogrel 300-600 mg loading dose, followed by a 75 mg daily for up to 12 months
   (b) Ticagrelor 180 mg loading dose, followed by 90 mg twice daily for up to 12 months

4. Comparison groups-CYP2C19 genes

<table>
<thead>
<tr>
<th>Dichotomous classification</th>
<th>Associated alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>No loss-of-function allele</td>
<td>None of the above alleles</td>
</tr>
</tbody>
</table>

5. Primary endpoint
   (a) Composite death from cardiovascular causes, myocardial infarction, or stroke

6. Secondary and safety endpoints
   (a) Composite death from cardiovascular causes and myocardial infarction
   (b) Stent thrombosis
   (c) Total major bleeding
   (d) Total major bleeding related to non-CABG
   (e) Total major bleeding related to CABG
   (f) Net clinical benefit – cardiovascular death, myocardial infarction, stroke, major bleeding related to non-CABG, and major fatal or life threatening bleeding related to CABG

7. Statistical analysis
   (a) Cox regression was used to compare outcomes between treatment groups and between genotype groups
   (b) Adjustment was made for the following covariates: ethnic group, sex, use of a proton-pump inhibitor, aspirin dose, smoking status, and diabetes
   (c) Kaplan-Meier estimates of the cumulative risk to the first occurrence of outcome events were calculated and plotted
   (d) Two-sided P values of less than 0.05 were considered significant

iii. Results
1. Baseline characteristics
   (a) 10285 patients genotyped, 5148 in the clopidogrel and 5137 in the ticagrelor group
   (b) No significant differences between groups
   (c) Mean age = 62.5 years
   (d) 30.7% were female, 69.3% male
   (e) 98% were Caucasian
   (f) 38% STEMI (clopidogrel group)
   (g) Approximately 60% received PCI, 18.9% received a drug-eluting stent (clopidogrel group)
   (h) 27% had at least one loss-of-function allele

2. Primary endpoint
   (a) The primary composite outcome of cardiovascular death, myocardial infarction, or stroke up to 12 months was consistently reduced with ticagrelor versus clopidogrel regardless of genotype group (P = 0.46 for interaction)

3. Secondary and safety endpoints
   (a) There were no significant differences between the genotype groups for any of the secondary or safety endpoints
   (b) Difference in stent thrombosis was not calculated between the genotype groups because of a low number of events
### Metabolizer phenotype

<table>
<thead>
<tr>
<th>Metabolizer phenotype</th>
<th>Ticagrelor events/total (%), Kaplan-Meier rate</th>
<th>Clopidogrel events/total (%), Kaplan-Meier rate</th>
<th>HR 95% CI</th>
<th>P value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any loss-of-function allele</td>
<td>115/1384 (8.3), KM-8.6%</td>
<td>149/1388 (10.7), KM-11.2%</td>
<td>0.77</td>
<td>0.60-0.99</td>
</tr>
<tr>
<td>No loss-of-function allele</td>
<td>296/3554 (8.3), KM-8.8%</td>
<td>332/3516 (9.4), KM-10.0%</td>
<td>0.86</td>
<td>0.74-1.01</td>
</tr>
</tbody>
</table>

4. Other analyses

   (a) Clopidogrel group only

      (i) When comparing event rates of the primary outcome within the clopidogrel group, those with any loss-of-function allele had a significantly higher rate at 30 days than those with none

         1. HR 1.37; 95% CI, 1.04–1.82 (P = 0.028)

         2. Analysis of the complete follow-up period found no significant difference between these groups

      (ii) For major bleeding events, patients in the clopidogrel group who had any gain-of-function allele had a higher rate of events than did those without any gain-of-function or loss-of-function alleles (P = 0.022)

   (b) Ticagrelor group only

      (i) The event rates between the two genotype groups in the ticagrelor arm were not significantly different and nearly identical

iv. Author’s conclusion

   1. “Ticagrelor is a more efficacious treatment for acute coronary syndromes than is clopidogrel, irrespective of CYP2C19 and ABCB1 polymorphisms. Use of ticagrelor instead of clopidogrel eliminates the need for presently recommended genetic testing before dual antiplatelet treatment.”

v. Critique

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Weaknesses</th>
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</thead>
<tbody>
<tr>
<td>- Effect modification design controls for sources of confounding, such as possible pleiotropic genetic effects</td>
<td></td>
</tr>
<tr>
<td>- Large trial</td>
<td></td>
</tr>
<tr>
<td>- No significant differences in baseline characteristics</td>
<td></td>
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<tr>
<td>- Included genotyping for many CYP2C19 genetic variants (not just the *1, *2, *3, and *17 alleles)</td>
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<tr>
<td>- Appropriate primary endpoint</td>
<td>- Retrospective analysis</td>
</tr>
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<td></td>
<td>- 98% white population</td>
</tr>
<tr>
<td></td>
<td>- <strong>Compliance</strong>?</td>
</tr>
<tr>
<td></td>
<td>- Changed from original intention to compare between six phenotype groups to just two genotype groups</td>
</tr>
<tr>
<td></td>
<td>- Differences is stent thrombosis not analyzed</td>
</tr>
</tbody>
</table>
c. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis

i. Methods

1. Study selection (includes TRITON-TIMI 38, CURE, PLATO, CHARISMA, and ACTIVE A substudies)

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic studies involving clopidogrel and CYP2C19</td>
<td>Controls not representative of the population from which cases were drawn</td>
</tr>
<tr>
<td></td>
<td>Case-reports</td>
</tr>
<tr>
<td></td>
<td>Clinical outcomes not measured</td>
</tr>
</tbody>
</table>

2. Comparison groups
   (a) Carriers of one or more reduced-function allele vs. noncarriers

3. Primary outcome
   (a) Composite all-cause mortality, CHD, Stroke, stent thrombosis, target vessel revascularization, and hospitalization for ACS
      (i) Assessed via “treatment only” analysis

4. Secondary outcomes
   (a) Stent thrombosis
   (b) Fatal and nonfatal MI and stroke
   (c) All cause mortality

ii. Results

1. Baseline characteristics
   (a) 26251 patients from 26 studies contributed to the primary outcome
   (b) Included studies in patients with ACS (managed conservatively or invasively), stable CHD, and atrial fibrillation
   (c) About 30% had at least one reduced-function allele

2. Primary and secondary outcomes (of note)

<table>
<thead>
<tr>
<th>Carriers of a reduced-function allele vs. noncarriers</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite endpoint</td>
<td>1.18 (1.09-1.28)</td>
</tr>
<tr>
<td>Stent thrombosis</td>
<td>1.75 (1.50-2.03)</td>
</tr>
<tr>
<td>Fatal and nonfatal MI</td>
<td>1.37 (1.13-1.65)</td>
</tr>
<tr>
<td>Nonfatal MI</td>
<td>1.48 (1.05-2.07)</td>
</tr>
<tr>
<td>Fatal and nonfatal Stroke</td>
<td>2.69 (0.55-13.29) †</td>
</tr>
<tr>
<td>All cause mortality</td>
<td>1.56 (0.92-2.64) †</td>
</tr>
</tbody>
</table>

*Using random-effects modeling; all other results presented from fixed-effects models

3. Other analyses
   (a) Evidence of small-study bias was found
      (i) Harbord test positive (P = 0.001)
   (b) Limiting analysis to studies with ≥ 200 events corrected the bias
      (i) Reduced analysis to only four studies including CURE and PLATO analyses
      (ii) RR 0.97; 95% CI, 0.86-1.28

d. Refuting evidence implications

i. Effects of CYP2C19 reduced-function alleles on clopidogrel users as a whole (all indications) may not be clinically significant
   1. Particularly conservatively managed patients
ii. A link to stent thrombosis remains
Genetic testing, alternative strategies, and guidelines

VI. Genetic testing\textsuperscript{20,27}

a. Available tests to analyze CYP2C19 genotype
   i. TaqMan\textsuperscript{®} assay
   ii. AmpliChip\textsuperscript{®} CYP450
   iii. INFINITI\textsuperscript{TM} Analyzer assay

b. Cost
   i. $400-700

c. Coverage??

VII. Alternative strategies

a. Increased clopidogrel dose\textsuperscript{28,29}
   i. A loading dose of 900 mg or a maintenance dose of 225 mg have been shown to overcome resistance in carriers of one CYP2C19 reduced-function allele but not two reduced-function alleles
   1. Assessed via platelet activity, not clinical outcomes

b. Prasugrel\textsuperscript{30}
   i. Rapidly hydrolyzed to active metabolite
   ii. CYP variants not shown to affect PK/PD or clinical outcomes

c. Ticlopidine\textsuperscript{31}
   i. Not shown to be dependent on CYP2C19 status

d. Ticagrelor\textsuperscript{25}
   i. Directly binds to platelets without need of activation
   ii. Not shown to be affected by CYP2C19 status

e. Cilostazol + clopidogrel\textsuperscript{32}
   i. Reduces platelet reactivity in CYP2C19 reduced-function allele carriers but not noncarriers

VIII. Guidelines

a. Clinical Pharmacogenetics Implementation Consortium Guidelines\textsuperscript{2}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7.png}
\caption{Figure 7. Scott SA, et al. Clin Pharmacol Ther 2011;90(2):328-332}
\end{figure}
b. **ACCF/AHA**
   i. Adherence to current guidelines for antiplatelet therapy recommended
   ii. Insufficient evidence to recommend routine genetic testing
   iii. Patients at moderate or high risk for poor outcomes which may include, among others, those undergoing elective high-risk PCI procedures (e.g., treatment of extensive and/or very complex disease)
      1. Testing may be considered
      2. If a poor metabolizer is identified, alternative therapy should be considered

**IX. Ongoing trials**

a. **Thrombocyte activity reassessment and genotyping for PCI (TARGET-PCI)**
   i. Prospective RCT
   ii. Treatment naive patients
      1. Therapy guided by CYP2C19 testing (clopidogrel or prasugrel)
         (a) Verigene® test
         (b) Run time about 2.5 hours
   iii. Primary endpoint:
      1. Composite of CV death, MI, stroke, or urgent target vessel revascularization

b. **Genotype guided comparison of clopidogrel and prasugrel outcomes study (GeCCO)**
   i. Prospective, cohort, non-inferiority trial
   ii. Comparing clopidogrel in those who are CYP2C19 extensive metabolizers to prasugrel
   iii. Primary endpoint:
      1. Composite of CV death, MI, or stroke

**X. Conclusion**

a. Clopidogrel non-responsiveness is associated with increased risk of recurrent cardiovascular events
b. CYP2C19 reduced-function alleles are associated with decreased metabolite exposure and antiplatelet effects

c. **Do CYP2C19 reduced-function alleles have an effect on clinical outcomes?**
   i. Patients receiving PCI
      1. Yes, based on evidence from the TRITON-TIMI 38 substudy/Mega et al, 2010 meta-analysis (> 90% PCI)
      ii. Conservatively managed patients or the entire population of clopidogrel users as a whole
      1. No, based on evidence from the CURE substudy (18% PCI)/Holmes et al, 2011 meta-analysis

d. **Should we be routinely testing for genetic variants?**
   i. No, to date there has not been any evidence showing that prospectively testing for CYP2C19 genotype improves outcomes in those receiving clopidogrel therapy

<table>
<thead>
<tr>
<th>ACC-American College of Cardiology</th>
<th>CKD-chronic kidney disease</th>
<th>NSTEMI-non-ST-elevation myocardial infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCF-American College of Cardiology Foundation</td>
<td>CV-cardiovascular</td>
<td>OR-odds ratio</td>
</tr>
<tr>
<td>ACS-acute coronary syndrome</td>
<td>CVA-cerebrovascular accident</td>
<td>PAD-peripheral artery disease</td>
</tr>
<tr>
<td>ADP-adenosine diphosphate</td>
<td>CYP-cytochrome P450</td>
<td>PCI-percutaneous coronary intervention</td>
</tr>
<tr>
<td>AHA-American Heart Association</td>
<td>DES-drug-eluting stent</td>
<td>PD-pharmacodynamic</td>
</tr>
<tr>
<td>ASA-American Stroke Association</td>
<td>EKG-electrocardiogram</td>
<td>PK-pharmacokinetic</td>
</tr>
<tr>
<td>AUC-area under the curve</td>
<td>EM-extensive metabolizer</td>
<td>PM-poor metabolizer</td>
</tr>
<tr>
<td>BMI-body mass index</td>
<td>GP-glycoprotein</td>
<td>RCT-randomized, controlled trial</td>
</tr>
<tr>
<td>CABG-coronary-artery bypass graft</td>
<td>HR-hazard ratio</td>
<td>STEMI-ST-elevation myocardial infarction</td>
</tr>
<tr>
<td>CAD-coronary artery disease</td>
<td>IM-intermediate metabolizer</td>
<td>TIA-transient ischemic attack</td>
</tr>
<tr>
<td>CHD-coronary heart disease</td>
<td>IPA-inhibition of platelet aggregation</td>
<td>UA-unstable angina</td>
</tr>
<tr>
<td>CHF-congestive heart failure</td>
<td>KM-Kaplan-Meier</td>
<td>UM-ultrarapid metabolizer</td>
</tr>
<tr>
<td>CI-confidence interval</td>
<td>MI-myocardial infarction</td>
<td></td>
</tr>
</tbody>
</table>
References


