New Insights in Stent Thrombosis

GENETICS AND STENT THROMBOSIS

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University of Verona
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Multifactorial Nature of DES Thrombosis

Device factors
- Surface
- Drugs
- Polymer
- Stent overlap

Procedural factors
- Dissection
- Incomplete stent apposition
- Stent expansion

Lesion factors
- Vessel size/length
- Thrombus
- Plaque characteristics
- Bifurcation
- Calcification
- Total occlusions

Patient factors
- Drug response/interactions
- Gene polymorphism
- LV function
- Acute coronary syndrome
- Renal failure
- Diabetes

Platelet and Coagulation factors
- Coagulation activity
- Inhibition of platelet aggregation
- Inadequate response to anti-platelet therapy
- Premature anti-platelet therapy discontinuation
Why genetics should be involved in stent thrombosis? Genetics is at the basis of any disease or disease response.

Previous genetic associations in interventional cardiology:

- In-stent restenosis
- Left ventricle hypertrophy
- Adrenergic receptors and coronary vasomotion
- Arrhythmic sudden death and long-QT syndrome
- Cardiomyopathies
- Hypertension, diabetes, lipids levels, myocardial infarction at young age …

DES: - DES thrombosis - AMI
Genetic associations in interventional cardiology: The case of Stent Thrombosis (ST)

Some of the most frequently quoted markers of ST:

Cytochrome P-450 Polymorphisms and Response to Clopidogrel


Vitamin K epoxide reductase complex subunit 1 gene polymorphism is associated with atherothrombotic complication after drug-eluting stent implantation: 2-Center prospective cohort study

Jung-Won Suh, MD, a Sang-Hong Baek, MD, PhD, a Jin-Sik Park, MD, a Hyun-Jae Kang, MD, a In-Ho Chae, MD, a Dong-Ju Choi, MD, a Han-Jun Park, MD, a Pu-Soon Kim, MD, a Ki-Bae Seung, MD, a and Hyo-Soo Kim, MD, PhD Seoul, South Korea
It was 1997, the study included 318 pts
F-up was 30 days
Genotyping was done with PCR
A single polymorphism analyzed

The hypotesis was made that IIb-IIIa GP inhibitors might reduce sub-acute stent thrombosis, in pts with the PI*A2 genotype
It was 2006, the study included 520 pts and 520 matched controls. Follow-up was 22 months. Genotyping was done with PCR. Single polymorphisms were analyzed.

There was no association between polymorphisms of factor V Leiden, prothrombin, or PAI-1 and cardiovascular events.

Plasma levels of PAI-1 and homocysteine yielded 5.3 and 7.5 odds ratio for occurrence of MACE.
764 pts in a prospective observation after DES implantation
F-up was 2 years

The TT genotype of a complex-sub-unit of the vitamin K yielded a lower risk of MACE compared to non TT type

HR: 2.56, 95%CI: 1.14-5.78, p=0.02

The authors suggested warfarin on top of DAT after DES implantation in non-TT patients
259 pts <45y old with a first MI under Clopidogrel treatment
F-up was 1 year

Genotyping of the CYP2C19 polymorphism

Carriers of the *2 “loss of function” allele had augmented risk of MACE:
HR: 3.69 95%CI: 1.69-8.05, p=0.0005,
MI: HR: 4.54 95%CI: 1.64-12.53, p=0.0001
and stent thrombosis: HR:6.02, 1.81-20.04, p=0.0009.
This was the only independent predictor of MACE: HR: 4.04, 1.81-9.02. p= 0.0006.

Authors suggest that the CYP2C19*2 “loss of function” genetic variant is a major determinant of prognosis in young MI pts under clopidogrel treatment.
A sub-analysis of the TRITON-TIMI 38

165 healthy subjects. Association between functional variants of the CYP gene and plasma concentrations of active drug metabolite Figure A or platelet inhibition in respond to clopidogrel Figure B.

The association of CYP2C19 variants and MACE was tested in 1477 pts with ACS under clopidogrel. Genotyping of the CYP2C19 polymorphism revealed 30% of carriers of the *2 “loss of function” allele. These had an augmented risk of MACE: HR: 1.53 1.7-2.09, p=0.01 and stent thrombosis: HR:3.09, 1.18-8.0, p=0.02.
Genetic Determinants of Response to Clopidogrel and Cardiovascular Events

Tabassome Simon, M.D., Ph.D., Céline Verstuyft, Pharm.D., Ph.D., Murielle Mary-Krause, Ph.D., Lina Quteineh, M.D., Elodie Drouet, M.Sc., Nicolas Méniveau, M.D., P. Gabriel Steg, M.D., Ph.D., Jean Ferrières, M.D., Nicolas Danchin, M.D., Ph.D., and Laurent Becquemont, M.D., Ph.D., for the French Registry of Acute ST-Elevation and Non–ST-Elevation Myocardial Infarction (FAST-MI) Investigators

2208 consecutive pts with AMI and under Clopidogrel treatment (FAST-MI Registry). Follow-up: 1y for MACE: death, MI, or stoke.

Association with allelic variants of CYP genes modulating:

**Clopidogrel absorption**: (ABCB1)
**Metabolic activation**: (CYP3A5-CYP2C19)
**Biological activity**: (P2RY12-ITGB3).

Carriers of the CYP2C19 *2 “loss of function” allele had a higher MACE rate: HR: 1.98, 1.10-5.58 and this risk was increased among pts undergoing PCI: 3.54, 1.71-7.51.
Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention


Clinical outcome

2465 pts under clopidogrel after stenting, prospectively assessed for ST at 30 days

27% were carriers of the *2 allele and had a HR: 3.81, 1.45-10.02, p=0.007 for ST. 1.5 vs 0.4%

Multivariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2 allele carriage</td>
<td>3.86 (1.47–10.14)</td>
<td>0.006</td>
</tr>
<tr>
<td>Age*</td>
<td>1.10 (0.69–1.77)</td>
<td>0.69</td>
</tr>
<tr>
<td>ACS</td>
<td>2.18 (0.69–6.84)</td>
<td>0.18</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.13 (0.82–5.58)</td>
<td>0.12</td>
</tr>
<tr>
<td>Type of stent</td>
<td>0.79 (0.23–2.76)</td>
<td>0.71</td>
</tr>
<tr>
<td>Use of abciximab</td>
<td>0.71 (0.27–1.87)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Clinical outcome

<table>
<thead>
<tr>
<th>Ischaemic events, n (%)</th>
<th>CYP2C19 *1/*1 (n = 1805)</th>
<th>CYP2C19 *1/*2 or *2/*2 (n = 680)</th>
<th>Hazard ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite ST</td>
<td>7 (0.4)</td>
<td>10 (1.5)</td>
<td>3.81 (1.45–10.02)</td>
<td>0.007</td>
</tr>
<tr>
<td>Death</td>
<td>16 (0.9)</td>
<td>5 (0.7)</td>
<td>0.83 (0.30–2.26)</td>
<td>0.71</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>111 (6.1)</td>
<td>48 (7.1)</td>
<td>1.15 (0.82–1.61)</td>
<td>0.42</td>
</tr>
<tr>
<td>STEMI</td>
<td>9 (0.5)</td>
<td>10 (1.5)</td>
<td>2.96 (1.20–7.28)</td>
<td>0.02</td>
</tr>
<tr>
<td>NSTE-ACS</td>
<td>102 (5.6)</td>
<td>38 (5.6)</td>
<td>0.99 (0.68–1.44)</td>
<td>0.96</td>
</tr>
<tr>
<td>Combined death/MI</td>
<td>121 (6.7)</td>
<td>52 (7.6)</td>
<td>1.14 (0.83–1.58)</td>
<td>0.42</td>
</tr>
<tr>
<td>Ischaemic stroke</td>
<td>0 (0.0)</td>
<td>4 (0.6)</td>
<td>-</td>
<td>0.001</td>
</tr>
</tbody>
</table>
All these studies confirm the role of a genetic variant modulating the response to Clopidogrel.

Interestingly, this is NOT a correlation between a genetic marker and a disease but an indirect demonstration of “adverse clinical events” mediated through a “lower than expected” pharmacogenetic response to a drug treatment focused on P2Y\textsubscript{12} receptors inhibitors.
The formation of active metabolites of thienopyridines pro-drugs (ticlopidine, clopidogrel and prasugrel) requires CYP450 metabolism. In particular CYP2C19 enzyme contributes to most of the clopidogrel activation, and therefore, defective genetic variants are associated with decreased plasma concentrations of the active metabolite.

Pharmacokinetic and pharmacodynamic studies indicate that the metabolism of prasugrel is not impacted by CYP450 polymorphisms.

**Figure 3** Major pathways leading to the formation of thienopyridine active metabolites.
Pharmacogenetics of P2Y₁₂ inhibitors

Cytochrome P450 enzymes

Clopidogrel → CYP2C19 → Active metabolite

Cytochrome P450 and proton pump inhibitors

Omeprazole is considered to have a higher potential for drug-drug interaction because of its capability to inhibit CYP2C19 activity and therefore reducing the bio-availability of the clopidogrel active metabolite

Augmented risk of stent thrombosis
Clopidogrel and PPIs: The OCLA study.

Clopidogrel is a prodrug; requires conversion by the liver primarily via CYP3A4 and CYP2C19 to an active metabolite.

PPIs are strong inhibitors of CYP2C19 activity.

PRI: Platelet Reactivity Index as measured by vasodilator stimulated phosphoprotein (VASP)

- Omeprazole (n=64): -32.6
- Placebo (n=60): -43.3

p<0.0001

Risk of All-Cause Mortality and Recurrent ACS in Patients Taking Clopidogrel and PPI

- Neither clopidogrel nor PPI
- PPI without clopidogrel
- Clopidogrel + PPI
- Clopidogrel without PPI

ORs for MACE according to the CYP2C19*2 allele and PPI use

Association between CYP2C19*2 allele and MACE (11959 pts).

MACE: 9.7 vs 7.8%,
OR: 1.29; 95%CI: 1.12-1.49, p<0.001.

Death: 1.8 vs 1.0%,
OR: 1.79; 95%CI: 1.10-2.91, p<0.02.

ST: 2.9 vs 0.9%,
OR: 3.45; 95%CI: 2.14-5.57, p<0.001.

Association between PPI use and MACE (48674 pts).

MACE: 21.8 vs 16.7%,
OR: 1.41; 95%CI: 1.34-1.48, p<0.001.

Death: 12.7 vs 7.4%
OR: 1.18; 95%CI: 1.07-1.30, p<0.001.
Analyses from TRITON-TIMI 38 and PRINCIPLE TIMI 44 do not confirm such previous observations.
The COGENT Trial

Deepak L. Bhatt MD, MPH, Byron Cryer MD, Charles F. Contant PhD, Marc Cohen MD, Angel Lanas MD, DSc, Thomas J. Schnitzer MD, PhD, Thomas L. Shook MD, Pablo Lapuerta MD, Mark A. Goldsmith, MD, PhD, Benjamin M. Scirica MD, Robert P. Giugliano MD, Christopher P. Cannon MD,

on Behalf of the COGENT Investigators
Methods

- Multicenter, international, randomized, double-blind, double-dummy, placebo-controlled, parallel group, phase 3 efficacy and safety study of CGT-2168, a fixed-dose combination of clopidogrel (75 mg) and omeprazole (20 mg), compared with clopidogrel.

- Patients were stratified based on two baseline factors: _H. pylori_ serology (positive or negative) and concomitant use of any NSAID.

- All patients were to receive enteric coated aspirin at a dose of 75 to 325 mg.
Survival Curves for PPI Treated vs Placebo Composite Cardiovascular Events

HR = 1.02
95% CI = 0.70; 1.51

Treated: 69 events, 1806 at risk
Placebo: 67 events, 1821 at risk

Adjustment through Cox Proportional Hazards Model
Adjusted to Positive NSAID Use and Positive H. Pylori Status
Survival Curves for PPI Treated vs Placebo
MI Events

HR = 0.96
95% CI = 0.59; 1.56

Placebo:
37 events, 1851 at risk
Treated:
36 events, 1839 at risk

Adjustment through Cox Proportional Hazards Model
Adjusted to Positive NSAID Use and Positive H. Pylori Status
Survival Curves for PPI Treated vs Placebo
Composite GI Events

HR = 0.55
95% CI = 0.36; 0.85
p=0.007
(preliminary)

Placebo: 67 events, 1895 at risk
Treated: 38 events, 1878 at risk
Conclusions

- COGENT is the first, randomized assessment of clopidogrel and PPIs on clinical events.

- The data provide strong reassurance that there is no clinically relevant adverse cardiovascular interaction between clopidogrel and PPIs.

- The results call into question the exact relationship between ex vivo platelet assays and clinical outcomes, especially with respect to assessing drug interactions.
  - Platelet assays and observational data are not a substitute for RCT data.

- Further research is needed to define the optimal strategy to reduce GI events in patients on antithrombotic therapy, though prophylactic PPIs seem very promising.
Multifactorial Nature of DES Thrombosis

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- Surface
- Drugs
- Polymer
- Stent overlap

Patient factors
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- LV function
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- Calcification
- Total occlusions

Platelet and Coagulation factors
- Coagulation activity
- Inhibition of platelet aggregation
- Inadequate response to anti-platelet therapy
- Premature anti-platelet therapy discontinuation
Predictors of Thrombosis After Successful Implantation of DES

- Diabetes: 2.5%
- Unprotected LMCA: 3.3%
- Bifurcation lesion: 3.6%
- Bifurcation with 2 stents: 3.9%
- Renal failure: 6.2%
- Prior brachytherapy: 8.7%
- Premature Antiplatelet Therapy Discontinuation: 20.4%
Recurrent very late drug-eluting stent thrombosis

Gabriele Pesarini, Mario Arieti, Roberta Spadaro, Corrado Vassanelli, Flavio Ribichini*

Division of Cardiology of the Ospedale Civile Maggiore, Department of Biomedical Sciences and Surgery of the University of Verona, 37126 Verona, Italy
Stent underexpansion with severe malapposition (red arrows).

Flow between the stent and the vessel wall.

Vessel wall calcification and stent indentation (yellow arrow).
Delayed Healing and Increased Inflammation at Sites of Drug-Eluting Stent Overlap

Joner et al.
JACC 2006 (N=32)

Finn et al.
Circulation 2007 (N=46)

Finn A et al. Circulation 2005;112:270-8
New Insights in Stent Thrombosis

GENETICS AND STENT THROMBOSIS

Genetics plays a role, but so far, a direct genetic-“cause-effect” is not established and needs further study.

Compliance to DAT, stent implantation technique and potential DES toxicity still account for most of the risk of stent thrombosis.
Future developments indicate routine genotyping as a “point of care” for DAT to offer either higher doses or different drugs to patients at risk.
However, this opinion needs *prospective* clinical trials to test the real efficacy of personalized anti-platelet therapy.
New Insights in Stent Thrombosis

GENETICS AND STENT THROMBOSIS

The future of personalized medicine... the genome-wide association studies and whole-genome sequencing

Challenges in the clinical application of whole-genome sequencing

*Kelly E Ormond, Matthew T Wheeler, Louanne Hudgins, Teri E Klein, Atul J Butte, Russ B Altman, Euan A Ashley, Henry T Greely*

The personal genome—the future of personalised medicine?

*Lancet 2010;375:1749-51
Lancet 2010;375: 1497-98*
One healthy man with family history of CAD and sudden death.

Genome sequencing (2.6 million SNPs) yielded augmented risk for MI, diabetes, osteoarthritis and some cancers…
One healthy man with family history of CAD and sudden death.

Genome sequencing (2.6 million SNPs) yielded .................
When the wise man points at the moon, the idiot looks at the finger (Confucius)
THANK YOU
Defining Stent Thrombosis

- Clinical syndrome
- **Acute** occlusion of a previously patent stent
- Not new plaque rupture at distant site
- Not severe restenosis with final occlusion
- Presents with acute coronary syndrome or sudden death
Premature Discontinuation of Thienopyridine Therapy After DES Implantation

Multicenter, prospective PREMIER registry in patients admitted with myocardial infarction
- 500 DES patients enrolled at 19 sites
- 68 (14%) patients d/c thienopyridine

Factors associated with premature Thienopyridine discontinuation
- older age
- lower socioeconomic status
- preexisting cardiovascular disease
- inadequate discharge instructions
- lack of referral to cardiac rehab

Mortality Between 30 Days and 1 Year

<table>
<thead>
<tr>
<th></th>
<th>Death</th>
<th>Rehosp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off Thienopyridine</td>
<td>7.5</td>
<td>0.7</td>
</tr>
<tr>
<td>On Thienopyridine</td>
<td>0.7</td>
<td>14</td>
</tr>
</tbody>
</table>

HR = 1.5
P = 0.08
HR = 9.0
P < 0.001
Clopidogrel: Double vs Standard Dose
Primary Outcome: PCI Patients

CV Death, MI or Stroke

- Clopidogrel Standard
- Clopidogrel Double (7 days)

15% RRR

HR 0.85
95% CI 0.74-0.99
P=0.036
Clopidogrel: Double vs Standard Dose
Definite Stent Thrombosis (Angio confirmed)

Clopidogrel Standard Dose

Clopidogrel Double Dose
(7 days)

Cumulative Hazard

Days

HR 0.58
95% CI 0.42-0.79
P=0.001

42% RRR
Conclusions
Clopidogrel Dose Comparison

1. Double-dose clopidogrel significantly reduced stent thrombosis and major CV events (composite of CV death, MI or stroke) in PCI.

2. In patients not undergoing PCI, double dose clopidogrel was not significantly different from standard dose (70% had no significant CAD or stopped study drug early for CABG).

3. There was a modest excess in CURRENT-defined major bleeds but no difference in TIMI major bleeds, ICH, fatal bleeds or CABG-related bleeds.
Heterogeneity of neointimal healing after DES placement and the impact on late stent thrombosis

<table>
<thead>
<tr>
<th></th>
<th>DES Thrombosis (n=28)</th>
<th>No DES Thrombosis (n=34)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrin score</td>
<td>2.4±1.3</td>
<td>1.2±1.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Endothelialization, %</td>
<td>40.5±29.8</td>
<td>80.8±25.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Uncovered strut per section, #</td>
<td>5.0±2.7</td>
<td>2.0±2.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stent length w/o neointima, mm</td>
<td>20.1±11.5</td>
<td>9.9±10.1</td>
<td>0.0004</td>
</tr>
<tr>
<td>Ratio of uncovered struts per total struts per section*</td>
<td>0.50±0.23</td>
<td>0.19±0.25</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*The most powerful morphometric predictor of endothelialization was RUTSS. The odds ratio for LST in lesions having an RUTSS >30% is 9.0 (95% CI, 3.5 to 22.0) with a sensitivity and specificity of 75% and 76%, respectively.
Bifurcation as Predictor of Stent Thrombosis

- OR=12.9 (4.7-35.8)
- OR=6.4 (2.9-14.1)
- OR=4.4 (2.0-10.0)
- OR=2.4 (1.1-5.6)

Odds Ratio

- Ong et al JACC 2005 in AMI
- Iakovou et al JAMA 2005
- Kuchulakanti et al Circulation 2006
- Roy et al J Interv Card 2007

Joner et al JACC 2006
Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants

Myocardial Infarction Genetics Consortium*

Table 4 Quintiles of allelic dosage score comprised of nine validated SNPs and risk for early-onset myocardial infarction

<table>
<thead>
<tr>
<th>Quintile of myocardial infarction genotype score</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quintile 1 (reference group)</td>
<td>1.0</td>
<td>1.04–1.44</td>
</tr>
<tr>
<td>Quintile 2</td>
<td>1.22</td>
<td>1.04–1.44</td>
</tr>
<tr>
<td>Quintile 3</td>
<td>1.43</td>
<td>1.22–1.68</td>
</tr>
<tr>
<td>Quintile 4</td>
<td>1.69</td>
<td>1.44–1.99</td>
</tr>
<tr>
<td>Quintile 5</td>
<td>2.23</td>
<td>1.89–2.63</td>
</tr>
</tbody>
</table>

P for association of myocardial infarction genotype score with early-onset myocardial infarction: $2 \times 10^{-28}$

The nine validated myocardial infarction polymorphisms are as shown in Table 2 and Table 3 and include SLC5A3-MRPS6-KCNE2 rs9982601, PHACTR1 rs12526453, WDR12 rs6725887, 9p21.3 rs4977574, CXCL12 rs1746048, CELSR2-PSRC1-SORT1 rs646776, MIA3 rs17465637, LDLR rs1122608, and PCKS9 rs11206510. Risk of early-onset myocardial infarction was assessed in the 2,967 cases and 3,075 controls from stage 1.