Effect of *PON1* and *CYP2C19* genetic variants on the pharmacokinetics and pharmacodynamics of clopidogrel

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Disclosures

• I have served as an expert and a consultant several private and public companies and institutions:
  – European Commission, Brussels, DG Research
  – National Heart, Lung and Blood Institute, Bethesda, USA
  – Medco Health Solutions
  – Biotronik

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  – Agence National pour la Recherche
  – Fédération Française de Cardiologie
  – Medco Health Institute
  – Biotronik
CYP2C19 & Metabolic activation of Clopidogrel

- There is a large degree of inter-individual variability in the response to clopidogrel
- Carriers of reduced-function CYP2C19 genetic variants have lower active metabolites levels, diminished on-treatment platelet inhibition and higher rates of MACE with a gene-dose effect (Mega et al. JAMA 2010; Hulot et al. JACC 2010)
- CYP2C19 only accounts for a limited proportion of this response variability
**PON1 as a new determinant of clopidogrel bioactivation**

- Paraoxonase-1 (PON1) is an hepatic esterase associated with high-density lipoprotein with a suspected role in atherosclerosis (Bhattacharyya, JAMA 2008)
- PON1 activity and level are influenced by two common non-synonymous polymorphisms (Q192R and L55M)
- Using *in vitro* liver microsomial assays, PON1 has recently been proposed in clopidogrel activation
- PON1 Q192 allele has been associated with lower clopidogrel active metabolite generation, lower platelet inhibition and higher rates of stent thrombosis

Bouman et al, Nat Med 2010

→ No impact of CYP2C19?
→ Few data on PON1 L55M
AIMS

• AIM 1:
  – To assess the association between PON1 (Q192R & L55M) and CYP2C19 polymorphisms and the occurrence of adverse cardiovascular outcomes in clopidogrel-treated patients

  Presented on August 28th,
  12:15 am, Room Tunis – Zone F

• AIM 2:
  – To study the impact of PON1 on the generation of clopidogrel H4 active metabolite (PK) and platelet inhibition (PD) in CAD patients (secondary analysis of the CLOVIS-2 trial)
Methods & Study design

- 106 post-MI patients who agreed to participate to the CLOVIS-2 study (Collet et al JACC Cardiovasc Inter 2011; 4:392-402):
  - Aged >18 y and < 45 y at the time of first MI
  - Treated with Aspirin 75 mg/d and clopidogrel 75 mg/day
  - Cross-over design, randomized allocation to 300 / 900 mg clopidogrel loading dose
  - PK/PD evaluation for 6 hours

**Figure 1. The CLOVIS-2 Study Design**

*PD = pharmacodynamic; PK = pharmacokinetic.*
Pharmacological measurements

- **Pharmacokinetics**
  - Sampling time: Baseline, 1, 2, 4 and 6 hours following intake
  - Whole blood was immediately stabilized after venipuncture with 500 mM MPBr
  - Active metabolite isomer H4 (clopi-H4) quantified by a stereoselective assay with LC/MSMS (Tuffal G, Thromb Haemost 2011)
  - C max and AUC0-6 hours computed by non-compartmental method (Winnonlin Software)

- **Pharmacodynamics**
  - Sampling time: Baseline, 6 hours following intake
  - Light Transmission Aggregometry (20umol/L ADP, residual platelet aggregation) & Verifynow P2Y12 (PRU)
  - Relative change in platelet aggregation calculated as:
    \[
    \frac{(\text{aggregation at baseline} - \text{aggregation at 6h post LD})}{\text{(aggregation at baseline)}} \times 100
    \]

- **Pharmacogenetics**
  - Taqman Fluorescent Allelic discrimination (applied Biosystems)
  - PON1 : Q192R (672 A>G, rs 662) & L55M (260T>A, rs 854560)
  - CYP2C19*2
Clopidogrel active metabolite generation in CAD patients is influenced by *CYP2C19* but not by *PON1*

<table>
<thead>
<tr>
<th></th>
<th>300 mg</th>
<th>900 mg</th>
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<tr>
<td></td>
<td>AUC₀-₆</td>
<td>p-value</td>
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<tr>
<td><strong>PON1 A672T</strong></td>
<td></td>
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<tr>
<td>(Q192R)</td>
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<tr>
<td>AA (QQ) n=44/46</td>
<td>16.2±10.8</td>
<td>0.41*</td>
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<tr>
<td>AG (QR) n=44/45</td>
<td>17.8±9.7</td>
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<tr>
<td>GG (RR) n=15/15</td>
<td>17.6±13.8</td>
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<tr>
<td><strong>PON1 A260T</strong></td>
<td></td>
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<tr>
<td>(L55M)</td>
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<tr>
<td>AA (MM) n=55/56</td>
<td>17.8±11.3</td>
<td>0.45*</td>
</tr>
<tr>
<td>AT (ML) n=41/42</td>
<td>16.9±10.7</td>
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<tr>
<td>TT (LL) n=6/8</td>
<td>11.5±2.5</td>
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<tr>
<td><strong>CYP2C19 G681A</strong></td>
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<tr>
<td>(CYP2C19*2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (*1/*1) n=55/58</td>
<td>19.7±11.9</td>
<td>0.01</td>
</tr>
<tr>
<td>GA (*1/*2) n=41/41</td>
<td>15.0±9.0</td>
<td></td>
</tr>
<tr>
<td>AA (*2/*2) n=7/7</td>
<td>9.9±2.9</td>
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* Overall P-value, Kruskall Wallis test.
All pairwise comparisons are non-significant

- Same results when analyzing Cmax values
- Multivariate linear regression: *CYP2C19*2 is the only significant predictor of AUC₀₋₆ following both 300 and 900mg LD
- *CYP2C19*2 however only account for 8% in the variation of clopidogrel PK
- No significant interactions between PON1 and CYP2C19
No influence of *PON1* on clopidogrel pharmacodynamics

Relative change in platelet response to 300 mg (white) and 900 mg (gray) clopidogrel LD according to *PON1*:

- Same results were found for residual and maximal platelet aggregation after stimulation with ADP 20 umol/L
- Proportion of patients with high on-treatment platelet reactivity:
  - QQ192 33.3% vs QR192 34.3% vs RR 192 33.3%, p=0.98
  - LL55 38.3% vs LM55 31.2% vs MM55 14.3%, p=0.50
Influence of CYP2C19 on clopidogrel pharmacodynamics

Collet et al
JACC Cardiovasc Inter 2011;4:392-402

→ Multivariate linear regression: CYP2C19*2 is the only significant predictor of platelet response to 300 and 900mg LD irrespective of the platelet function assay

→ No significant interactions between PON1 and CYP2C19
No influence of \textit{PON1} on adverse cardiovascular outcomes in the AFIJI cohort

\textit{(Collet et al Lancet 2009)}

\textbf{MACE}

\begin{align*}
\text{HR} &= 0.84 \quad 95\% \text{CI}[0.43 \text{-} 1.67] \\
\text{p} &= 0.62
\end{align*}

\begin{align*}
\text{HR} &= 1.68 \quad 95\% \text{CI}[0.86 \text{-} 3.27] \\
\text{p} &= 0.13
\end{align*}

\rightarrow \text{Significant association between LL55 genotype and diabetes}
CYP2C19*2 carriers & higher rates of outcomes

Cox regression analysis considering PON1 and CYP2C19:

<table>
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<tr>
<th></th>
<th>Hazard Ratio (95%CI)</th>
<th>P-value</th>
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<tr>
<td>PON1 QQ192 vs QR/RR 192</td>
<td>1.03 [0.50-2.11]</td>
<td>0.93</td>
</tr>
<tr>
<td>PON1 LL55 vs LM/MM55</td>
<td>1.52 [0.75-3.08]</td>
<td>0.24</td>
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<tr>
<td>CYP2C19*2 carriers vs non-carriers</td>
<td>2.26 [1.15-4.41]</td>
<td>0.02</td>
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CONCLUSIONS

• The present results do not support *PON1* polymorphisms as major determinants of clopidogrel PK and PD responsiveness, including the clinical efficacy of clopidogrel in young post-MI patients.

• The generation of clopidogrel active metabolite isomer H4 *in vivo* depends upon CYP2C19 but not PON1.

• *PON1* polymorphisms might however be associated to the generation of other inactive metabolites.
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