Can $ABCB1$ genetic polymorphisms explain the inter-individual variability in DOAC plasma concentrations?
DOACs have a predictable dose response. They are given in a fixed-dose regimen.

Inter-individual variability in DOAC plasma concentrations has been described.

DOAC plasma levels in patients with AF during routine visits

Gulilat et al, Canadian Journal of Cardiology 2017; 33:1036-1043
P-glycoprotein (ABCB1, MDR1) Transporters that rely on ATP to actively pump substrates across cell membranes

- Paclitaxel, doxorubicin, etoposide
- Erythromycin, clarithromycin, rifampicin
- Ciclosporin, tacrolimus
- Imatinib, nilotinib, dasatinib
- Ritonavir, saquinavir
- Digoxin, quinidine, amiodarone
- Diltiazem, verapamil
- Atorvastatin, simvastatin
- Bisoprolol, clopidogrel
- Rivaroxaban, dabigatran etexilate
- Apixaban, edoxaban

42% of hospitalized patients with AF take P-gp affecting drugs.

**ABCB1 genetic polymorphisms**

Normal variations between individuals in their DNA sequence

Main source of genetic variability = single nucleotide polymorphisms (SNP)

1 SNP every 300 base pairs!

**ABCB1** Chromosome 7q –1279 SNPs (*62 coding SNPs*)

**ABCB1 genetic polymorphisms**

### 3 most common SNPs

<table>
<thead>
<tr>
<th>SNP</th>
<th>Exon</th>
<th>Amino Acid Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1236C&gt;T</td>
<td>Exon 13</td>
<td>Gly412Gly</td>
</tr>
<tr>
<td>2677G&gt;T/A</td>
<td>Exon 22</td>
<td>Ala893Ser/Thr</td>
</tr>
<tr>
<td>3435C&gt;T</td>
<td>Exon 26</td>
<td>Ile1145Ile</td>
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</table>

Allelic frequency around 50% in Caucasians
Strong linkage disequilibrium

**Other SNP of interest**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Exon</th>
<th>Amino Acid Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1199G&gt;A</td>
<td>Exon 11</td>
<td>Ser400Asn</td>
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</tbody>
</table>

Nonsynonymous SNP (amino acid change)
Cytoplasmic loop involved in substrate recognition
Allelic frequency around 6% in Caucasians

**Immunosuppressant, anticancer agents, anti-HIV, antiepileptic drugs, antidepressants...**

<table>
<thead>
<tr>
<th>Compound</th>
<th>1199A activity* (in vitro assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine</td>
<td>= or ↓ (fluorescence)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>= (cytotoxicity)</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>↑ (cytotoxicity)</td>
</tr>
<tr>
<td>Vincristine</td>
<td>↑ (cytotoxicity)</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>↑ (cytotoxicity)</td>
</tr>
<tr>
<td>Etoposide (VP-16)</td>
<td>↑ (cytotoxicity)</td>
</tr>
<tr>
<td>HIV protease inhibitors</td>
<td>↑ (accumulation)</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>= or ↑ (accumulation)</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>↓ (accumulation)</td>
</tr>
</tbody>
</table>

Objectives and method

Can *ABCB1* genetic polymorphisms explain the inter-individual variability in DOAC plasma concentrations?

*in vitro* effect of *ABCB1* genetic polymorphisms on the transport activity towards DOACs

- **1236C>T-2677G>T-3435C>T**
  - Control = empty vector
  - 1236C, 2677G, 3435C
  - 1236C, 2677G, 3435T
  - 1236T, 2677T, 3435T

- **1199G>A**
  - Control = empty vector
  - 1199G
  - 1199A

HEK293 cells (Human Embryonic Kidney) Stably transfected to overexpress ABCB1

Intracellular accumulation Rivaroxaban
**INTRODUCTION**

**OBJECTIVES-METHOD**

**RESULTS**

**DISCUSSION**

1. Cell culture
2. ABCB1 expression
3. Intracellular accumulation
4. LC-MS/MS analysis

<p>| | | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>D1</td>
<td>± D10</td>
<td>± D14</td>
<td>± D21</td>
</tr>
</tbody>
</table>

Cells in 24-well plates

Addition of rivaroxaban at 5 different concentrations:

- 50 ng/ml
- 100 ng/ml
- 250 ng/ml
- 500 ng/ml
- 1000 ng/ml

Triplicates

Incubation for 120 min

Centrifugation and removal of the supernatant

Conservation of cell pellets at -80°C until quantification
Intracellular accumulation of rivaroxaban after 120 min of incubation (N=3)

- **1236C>T-2677G>T-3435C>T**
  - Intracellular accumulation ↓ in recombinant cells overexpressing ABCB1
  - No statistical difference between cells overexpressing the ABCB1 WILD-TYPE and VARIANT proteins

- **1199G>A**
  - Compared to control cells: *p<0.05 **p<0.01 ***p<0.001
**ABCB1 polymorphisms and DOACs**

1st *in vitro* study on the role of ABCB1 genetic determinants in the transport of DOAC

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**Rivaroxaban-Induced Hemorrhage Associated with ABCB1 Genetic Defect**

Kuntheavy Ing Lorenzini1*, Youssef Daali2, Pierre Fontana2, Jules Desmeules1 and Caroline Samer1

---

79-year-old male – rivaroxaban 20mg OD
231 ng/ml (24h post-intake) + delayed clearance

**Role of 2677TT-3435TT ?**

↔ High frequency of the homozygous genotype
↔ Renal impairment on admission
↔ Concomitant use of simvastatin

---

60 Caucasian healthy males
No significant effect of 2677G>T-3435C>T on DOAC pharmacokinetics
Clarithromycin: ↑ AUC 2x

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**ORIGINAL ARTICLE**

*Interindividual variability in dabigatran and rivaroxaban exposure: contribution of ABCB1 genetic polymorphisms and interaction with clarithromycin*

How to explain the findings?

- **1199G>A has a substrate-dependent impact on drug transport.**
  
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</table>

  Structural flexibility and multiple binding sites of ABCB1

- **Rivaroxaban is a weak to moderate substrate for ABCB1.**

  Effect of verapamil (P-gp inhibitor, weak CYP34 inhibitor)

  Caco-2 cells: ↓ rivaroxaban efflux by 23%
  ↓ dabigatran etexilate efflux by 87%

- **Rivaroxaban is a substrate for the ABCG2 transporter (BRCP)**
  
  - Compensatory role of BCRP
  - ↓ rivaroxaban clearance in mice lacking both ABCB1 and ABCG2
Summary

**What is already known**

- P-glycoprotein is involved in the transport of all DOACs.
- *ABCB1* polymorphisms influence the transport activity towards several drugs.
- There is a high inter-individual variability in DOAC plasma concentrations.

**What this study adds**

- The intracellular accumulation of rivaroxaban was influenced by the overexpression of *ABCB1*.
- The *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs had no significant effect on the efflux of rivaroxaban in HEK293 cell lines.
- They are unlikely to contribute to the inter-individual variability in rivaroxaban plasma levels.
THANK YOU

Integrated PHarmacoMetrics, PharmacoGenomics and PharmacoKinetics (UCL, LDRI)
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Nadtha Panin
Francine Uwambayinema
Saloua Ibouraadaten

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Lionel Pochet
Christelle Vancraeynest
Romain Siriez

Funding
Fonds de la Recherche Scientifique - FNRS
All DOACs are moderate substrates for ABCB1. They are also substrate for ABCG2 (BCRP).

!! Dabigatran is not a P-gp substrate.
Active renal secretion by P-gp and BCRP = 30% of total rivaroxaban elimination
Characterization of ABCB1 cell surface expression by flow cytometry

in vitro effect of ABCB1 genetic polymorphisms

1236C>T-2677G>T-3435C>T
1199G>A

Characterization of ABCB1 cell surface expression by flow cytometry

II. *in vitro* effect of *ABCB1* genetic polymorphisms

Red = isotype control, blue = anti-ABCB1
## Genotyping in DOAC-treated patients

<table>
<thead>
<tr>
<th>N°</th>
<th>Age</th>
<th>Sexe</th>
<th>DOAC</th>
<th>ADR</th>
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<td>1</td>
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### Genotyping in DOAC-treated patients

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<th>SNP</th>
<th>Alleles</th>
<th>Observed</th>
<th>Expected</th>
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<tbody>
<tr>
<td>1236C&gt;T</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>AA</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

- **1236C>T**
  - Observed: 5, 10, 6
  - Expected: 7, 10, 4
  - $P^T$ observed = 0.52
  - $P^T$ expected = 0.42
  - $X^2$ = 0.46

- **2677G>T**
  - Observed: 6, 9, 6
  - Expected: 7, 10, 4
  - $P^T$ observed = 0.50
  - $P^T$ expected = 0.41
  - $X^2$ = 0.53

- **3435C>T**
  - Observed: 4, 9, 8
  - Expected: 5, 10, 6
  - $P^T$ observed = 0.60
  - $P^T$ expected = 0.52
  - $X^2$ = 0.62

- **1199G>A**
  - Observed: 20, 1, 0
  - Expected: 20, 1, 0
  - $P^A$ observed = 0.02
  - $P^A$ expected = 0.03

- **rs4148738**
  - Observed: 6, 10, 5
  - Expected: 4, 10, 7
  - $P^A$ observed = 0.48
  - $P^A$ expected = 0.56
  - $X^2$ = 0.46

Cut-off of significance at $\alpha=0.05$: 3.84