Pharmacogenetics of cytochrome P450 and its application and value in drug therapy – the past, present and future

Magnus Ingelman-Sundberg
Karolinska Institutet, Stockholm, Sweden
The human genome

- 3,120,000,000 nucleotides
- 23,000 genes
- >100,000 transcripts (!)
- up to 100,000 aa differences between two proteomes
- 10,000,000 SNPs in databases today

The majority of the human genome is transcribed and has an unknown function

RIKEN consortium Science 7 Sep 2005
Interindividual variability in drug action

Absorption / Excretion
- Slow
- Rapid
- Slow
- Rapid

Receptor interactions
- Poor
- Efficient

Metabolism
- Poor
- Efficient
- Ultrarapid

Drug–drug interactions

Drug–drug

Drug–food

interactions

Interactions

Kidney function

Ingelman-Sundberg, M., J Int Med 250: 186-200, 2001,
CYP dependent metabolism of drugs (80 % of all phase I metabolism of drugs)

Beta blockers
Antidepressants
Antipsychotics
Dextromethorphan
Codeine
Debrisoquine

CYP2D6*

CYP2C9*

CYP2C19*

Beta blockers
Antidepressants
Antipsychotics
Dextromethorphan
Codeine
Debrisoquine

CYP3A4/5/7
Cyclosporin
Taxol
Tamoxifen
Tacrolimus
Amprenavir
Amiodarone
Cerivastatin
Erythromycin
Methadone
Quinine

CYP2E1
CYP1A2
40 % of the phase I metabolism is carried out by polymorphic P450s (enzymes in Italics)

CYP2B6*

Tolbutamide
Warfarin
Phenytoin
NSAID
Diazepam
Citalopram
Anti ulcer drugs
Clozapine
Ropivacaine
Efavirenz
Cyclophosphamide
Phenotypes and mutations

PM, poor metabolizers; IM, intermediate met; EM, efficient met; UM, ultrarapid met

Frequency

- Phenotypes and mutations
  - Homozygous for
    - Stop codons
    - Deletions
    - Deleterious missense SNPs
    - Splice defects
  - Heterozygous deleterious SNPs
  - Unstable protein
  - Gene duplication
  - Induction

Population based dosing

Gene duplication

Enzyme activity/clearance

Population based dosing

PM, poor metabolizers; IM, intermediate met; EM, efficient met; UM, ultrarapid met
The Home Page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee

http://www.imm.ki.se/CYPalleles/

Webmaster: Sarah C Sim

Editors: Magnus Ingelman-Sundberg, Ann K. Daly, Daniel W. Nebert

Advisory Board: Jürgen Brockmöller, Michel Eichelbaum, Seymour Garte, Joyce A. Goldstein, Frank J. Gonzalez, Fred F. Kadlubar, Tetsuya Kamataki, Urs A. Meyer, David R. Nelson, Michael R. Waterman, Ulrich M. Zanger.

Nomenclature files for human cytochrome P450 alleles:
   CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2A13, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP2R1, CYP2S1, CYP3A4, CYP3A5, CYP3A7, CYP5A1, CYP8A1, CYP21.
Cytochrome P450s and ADRs

- 59% of drugs cited in ADR-studies are metabolised by polymorphic phase 1 enzymes - P450s account for 86% of those
- Only 20% of drugs which were substrates for nonpolymorphic enzymes were in the ADR reports
- CYP2D6 was involved in 38% of all ADR reports

CYP2D6 and rate of metabolism in the European population

Based on the European population with 7% PMs and 5.5% UMs overall

CYP2D6-based dose adjustments for antidepressants and antipsychotics

Kirchheiner et al., Mol Psychiatry 2004
CYP2D6 and the European population

20-30 million subjects have no CYP2D6 enzymes (PMs)

15-20 million subjects have CYP2D6 gene duplications (UMs)

resulting in

- Too slow drug metabolism
- Too high drug levels at ordinary dosage
- High risk for ADRs
- No response from certain prodrugs (e.g. codeine)

Relevant for 15% of all drugs used
Pharmacogenetics based dose adjustments: CYP2C9*3

Kirchheiner & Brockmöller, 2005
Warfarin dosage variation: 0.5-8 mg/day

CYP2C9

\downarrow

25% prediction

\downarrow

+ VKORC1

\downarrow

52% prediction

\downarrow

+ age and weight

\downarrow

62% prediction

VKOR = vit K epoxide reductase
Multiple regression model explains 61% of variance in warfarin dose

<table>
<thead>
<tr>
<th>Variables</th>
<th>Dose</th>
<th>p</th>
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<tbody>
<tr>
<td>VKORC1</td>
<td>&lt;.0001</td>
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</tr>
<tr>
<td>CYP2C9</td>
<td>&lt;.0001</td>
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<tr>
<td>PROC</td>
<td>0.0541</td>
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<tr>
<td>Age</td>
<td>0.0002</td>
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<tr>
<td>Bodyweight</td>
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</tr>
<tr>
<td>Indication</td>
<td>0.0406</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>0.1018</td>
<td></td>
</tr>
</tbody>
</table>

Total $r^2$ for the model = 0.614

Wadelius M, Chen LY, Eriksson N, Ghori J, Wadelius C, Bentley D, McGinnis R, Deloukas P. Uppsala University, Sweden and the Wellcome Trust Sanger Institute, UK.
A novel ultrarapid CYP2C19 allele (CYP2C19*17)

Ethiopians

Kruskal-Wallis p=0.018

Swedes

Kruskal-Wallis p=0.011
Frequency and effect of *CYP2C19*<sup>17</sup> on clinical parameters

<table>
<thead>
<tr>
<th>Population</th>
<th>Allele frequency</th>
<th>*1/*1</th>
<th>*1/*17</th>
<th>*17/*17</th>
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</thead>
<tbody>
<tr>
<td>Swedes</td>
<td>20.1</td>
<td>155</td>
<td>80</td>
<td>9</td>
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<tr>
<td>Ethiopians</td>
<td>17.9</td>
<td>126</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Tanzanians</td>
<td>16.3</td>
<td>63</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>Chinese&lt;sup&gt;*&lt;/sup&gt;</td>
<td>5.0</td>
<td>54</td>
<td>6</td>
<td>0</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>*17/*17</th>
<th>*1/*17</th>
<th>*1/*1</th>
<th>*1/*2</th>
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<tr>
<td>n</td>
<td>4</td>
<td>23</td>
<td>38</td>
<td>16</td>
</tr>
<tr>
<td>Omeprazole MR</td>
<td>0.25±0.10</td>
<td>0.44±0.44</td>
<td>0.77±0.70</td>
<td>1.17±0.75</td>
</tr>
<tr>
<td>Pred omeprazole AUC</td>
<td>742±93</td>
<td>926±410</td>
<td>1240±658</td>
<td>1620±710</td>
</tr>
<tr>
<td>Predicted intragastric pH</td>
<td>3.50</td>
<td>3.75</td>
<td>4.20</td>
<td>4.50</td>
</tr>
</tbody>
</table>
CYP3A locus: INTERETHNIC DIFFERENCES

### European
- 3A5
- 3A7
- 3A4
- 3A4 prom

- 3A5 rs776746, MAF = 5.8 (A)
- 3A7 rs1357319, MAF = 6.7 (A)
- 3A4 rs2242480, MAF = 8.3 (T)

### Chinese
- 3A5
- 3A7
- 3A4
- 3A4 prom

- 3A5 rs776746, MAF = 33 (A)
- 3A7 rs1357319, MAF = 34 (A)
- 3A4 rs2242480, MAF = 26 (T)

### African
- 3A5
- 3A7
- 3A4
- 3A4 prom

- 3A5 rs776746, MAF = 15 (G)
- 3A7 rs1357319, MAF = 35 (C)
- 3A4 rs2242480, MAF = 11 (C)

<table>
<thead>
<tr>
<th>Gene</th>
<th>MAF (%)</th>
<th>European</th>
<th>Chinese</th>
<th>Africans</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A5 rs776746</td>
<td>6 (A)</td>
<td>33 (A)</td>
<td>15 (G)</td>
<td></td>
</tr>
<tr>
<td>CYP3A7 rs1357319</td>
<td>7 (A)</td>
<td>34 (A)</td>
<td>35 (C)</td>
<td></td>
</tr>
<tr>
<td>CYP3A4 rs2242480</td>
<td>8 (T)</td>
<td>26 (T)</td>
<td>11 (C)</td>
<td></td>
</tr>
</tbody>
</table>
Polymorphically determined expression of CYP3A7 in human adult liver

- one in 10 adult livers expressed CYP3A7 at 24-90 pmol/mg (9-36% to total CYP3A levels in these livers).
- 5/7 livers with CYP3A7*1C expressed CYP3A7 protein.
- In 57 livers CYP3A7 was present at 4 pmol/mg, *higher* than that of CYP3A5.

### HAPLOTYPE

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Estimated Frequency (%)</th>
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</thead>
<tbody>
<tr>
<td>CYP3A7*1</td>
<td>Caucasians</td>
</tr>
<tr>
<td>CYP3A7*2</td>
<td>90</td>
</tr>
<tr>
<td>CYP3A5*3</td>
<td>7</td>
</tr>
<tr>
<td>CYP3A5*1</td>
<td>18.0</td>
</tr>
</tbody>
</table>

**CYP3A7 activity**

- **16α-OH DHEA**
  - CYP3A7.1
  - CYP3A7.2

**CYP3A5 activity**

- **αOH Alprazolam**
  - FL55
  - FL61
  - FL65

**DHEA (µM)**

- 0
- 25
- 50
- 75
- 100

**16α-OH DHEA Activity**

- (pmol min⁻¹ pmol P450⁻¹)

**αOH Alprazolam**

- (pmols min⁻¹ mg prot⁻¹)
# Genotyping for CYPs

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>substrates</th>
<th>phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2B6</td>
<td>cyclophosphamide, efavirenz</td>
<td>IM/EM</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>warfarin, antidiabetics, phenytoin, celecoxib</td>
<td>PM/IM/EM</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>antiulcer drugs, citalopram</td>
<td>PM/EM/UM</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>antidepressants, antipsychotics, codeine, tramadol, perhexiline, antiemetic drugs,</td>
<td>PM/IM/EM/UM</td>
</tr>
</tbody>
</table>
## Examples of clinical impact of cytochrome P450 pharmacogenetics

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme</th>
<th>UMs</th>
<th>PMs</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>CYP2C9</td>
<td></td>
<td></td>
<td>Bipolar disorders and valproate PMs and SSRIs</td>
</tr>
<tr>
<td></td>
<td>CYP2C19</td>
<td>40</td>
<td></td>
<td>Non-responders (UMs) and side effects of tricyclics (PMs)</td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>200</td>
<td>30</td>
<td>Non-responders (UMs) and side effects of tricyclics (PMs)</td>
</tr>
<tr>
<td>Psychosis</td>
<td>CYP2D6</td>
<td>160</td>
<td>30</td>
<td>Haloperidol and parkinsonian side effects</td>
</tr>
<tr>
<td>Ulcer</td>
<td>CYP2C19</td>
<td>20</td>
<td></td>
<td>Dosing of PPIs pH and gastrin changes</td>
</tr>
<tr>
<td>Cancer</td>
<td>CYP2B6</td>
<td></td>
<td></td>
<td>Cyclophosphamide metabolism</td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>250</td>
<td>60</td>
<td>Non-response of antiemetic drugs (UMs)</td>
</tr>
<tr>
<td>CV</td>
<td>CYP2C9</td>
<td>30</td>
<td></td>
<td>warfarin dosing (acenocoumarol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Irbesartan and blood pressure response; Perhexiline neuropathy and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hepatotoxicity,</td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>160</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>CYP2D6</td>
<td></td>
<td></td>
<td>Codeine no response (PMs)</td>
</tr>
<tr>
<td>Epilepsia</td>
<td>CYP2C9</td>
<td></td>
<td></td>
<td>Phenytoin pharmacokinetics and side effects</td>
</tr>
</tbody>
</table>
Conclusions, pharmacogenetics in future drug treatment

- In 15-25% of the cases of drug treatment genotyping will be very important for prediction of drug efficacy and drug toxicity.
- For certain drugs genotyping will be relevant in 100% of the cases.
- Predictive genotyping might prevent 10-20% of serious and fatal adverse drug reactions.
- In 15-35% drug metabolism is influenced by pylogenic factors and difficult to foresee by genotyping.
- In 50% of the cases genetic factors are of limited importance.
Drug Reaction Testing

Do not alter the dosage amount or schedule of any drug you are taking without first consulting your doctor or pharmacist.

Research shows that of all the clinical factors such as age, sex, weight, general health and liver function that alter a patient's response to drugs, genetic factors are the most important. This information becomes even more crucial when you consider the fact that adverse reactions to prescription drugs are killing about 106,000 Americans each year -- roughly three times as many as are killed by automobiles. This makes prescription drugs the fourth leading killer in the U.S., after heart disease, cancer, and stroke.

We currently offer CYP2D6, CYP2C9, CYP2C19, and CYP1A2 screens that can help your physician or pharmacist predict your particular response to many prescription, OTC (over-the-counter) and herbal medicines including those used to treat depression, anxiety, seizures and psychoses; blood pressure, anticoagulation and other heart medicines; anti-diabetic agents, and many pain relievers. These include such important medications as Coumadin (Warfarin), Prozac, Zoloft, Paxil, Effexor, Hydrocodone, Amitriptyline, Claritin, Cyclobenzaprine, Haldol, Metoprolol, Rythmol, Tagamet, Tamoxifen, Valium, Carisoprodol, Diasepam, Dilantin, Premarin, andPrevacid (and the over-the-counter drugs, Allegra, Dytuss and Tusstat). Click here to view a more complete list of drugs processed through these pathways.

Approximately half of all Americans have genetic defects that affect how they process these drugs. There are four different types of metabolizers, and we all fall into one of these categories for the variable pathways in
Future (cont…)

- Large prospective studies with well characterized patients on monotherapy
- Pharmacogenetics is used during drug development
- The urgent need concerns old drugs – industry will not finance such studies
- Pharmacogenetics is not used in the clinics unless required by regulations
- Development of guidelines of critical importance
- Implementation in the clinics after guidelines will occur
Specific genotype → Combine
Specific Drug