Genetics of Phase I Enzymes
Aiming Yu, Ph.D.

University at Buffalo
State University of New York
Department of Pharmaceutical Sciences
Buffalo, NY 14260, USA
E-mail: aimingyu@buffalo.edu

Outline

- Inter-patient variations and phase I drug-metabolizing enzymes
- The genetic polymorphism of CYP2D6 and its impact on psychopharmacotherapy
- Pharmacogenetics and pharmacogenomics move towards individualized drug therapy

Inter-Patient Variations in Drug Therapy

Drug treatment

Responses
- Normal (desired)
- No or minimal
- Exaggerated
- Adverse (toxic)

The screen versions of these slides have full details of copyright and acknowledgements.
Variations in Phase I Metabolism: Debrisoquine/Sparteine Phenotypes

- Severe side effects (orthostatic hypotension) reported by the volunteers taking debrisoquine at standard dose
- Serious side effect (nausea, diplopia, blurred vision) experienced by the volunteer taking sparteine at standard dose

Eichelbaum et al. (1979) Eur J Clin Pharmacol 16, 183

Metabolic Ratio (MR)

Debrisoquine/4-hydroxydebrisoquine


Causes of Inter-Patient Variations

- Genetic polymorphism
  - Receptor/enzyme/ion channel targets
  - Drug-metabolizing enzymes
  - Transporters
- Environmental factors
  - Diet, beverage, tobacco smoking, concomitant medications
- Miscellaneous
  - Age, sex, body weight, disease, etc.

Phase I Drug-Metabolizing Enzymes

- Cytochrome P450s (P450 or CYP)
- Flavin-containing monooxygenases (FMO)
- Monoamine oxidases (MAO)
- Epoxide hydrolases
- Thiopurine S-methyltransferase

R₁ R₂ → R₁ R₂ OH
Genetics of Human CYP Enzymes

- 57 functional CYP genes and 33 pseudogenes identified in humans
- Being classified in 17 families (>40% amino acid sequence identity) and 42 subfamilies (>55%)

**CYP2D6**
- Individual gene identified by a #
- Subfamily identified by a letter
- Cytochrome P450

- Enzymes belonging to CYP 1, 2, 3 and 4 families are important in drug metabolism

Genetic Polymorphism of CYP Enzymes

- Polymorphism in a gene is defined as the incidence of its mutant allele ≥ 1% in a population
- To name an allele, the allele and gene is separated by an asterisk followed by Arabic numerals and upper-case Roman letter
  - E.g. CYP2D6*4, CYP2D6*10B, CYP2C19*2

Clinically Important Polymorphic Human Phase I CYP Isozymes

<table>
<thead>
<tr>
<th>CYP</th>
<th>Psychotropic drug substrate</th>
<th>Frequency of PMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9</td>
<td>Phenytoin</td>
<td>1-3% Caucasians</td>
</tr>
<tr>
<td></td>
<td>Warfarin</td>
<td></td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Mephenytoin</td>
<td>3-5% Caucasians</td>
</tr>
<tr>
<td></td>
<td>Omeprazole</td>
<td>14-20% Asians</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Debrisoquine</td>
<td>5-10% Caucasians</td>
</tr>
<tr>
<td></td>
<td>Sparteine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antidepressants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antipsychotics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-Blockers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psychedelics</td>
<td></td>
</tr>
</tbody>
</table>
CYP2C19 Pharmacogenetics
- Major defective alleles: CYP2C19*2 and *3
- Psychotropic drug substrates: amitriptyline, citalopram, clomipramine, diazepam, imipramine, moclobemide
- Citalopram: differentiated PK in EMs and PMs, but clinical impact is unknown
- Diazepam: decreased clearance in PMs, but CYP3A4 metabolism may compensate; lack of evidence of altered pharmacodynamics
- Moclobemide: CYP2C19 inhibitor, omeprazole, alters the metabolism of moclobemide in EMs

Psychotropic Drugs
Metabolized by CYP2D6
- ~50% of all psychoactive drugs and
- ~35% of prescribed psychoactive drugs
- Antidepressants: amitriptyline, clomipramine, desipramine, fluoxetine, fluvoxamine, imipramine, nortriptyline, etc.
- Antipsychotics: haloperidol, perphenazine, risperidone, etc.
- Beta-blockers: metoprolol, propranolol, timolol, etc.
- Hallucinogens and stimulants: Phenylalkylamines (MDMA “ecstasy”), Indolealkylamines (5-MeODMT)
- Narcotics/analgesics: Codeine

Human CYP2D Gene Cluster
22p13 22p12 22q11.2 22q12 22q13
XbaI 29kb XbaI
1 2 3 4 5 6 7 8 9 3'
5' 2D8P 2D7P 2D6
Pseudogenes Functioning Gene 22q13.1

The screen versions of these slides have full details of copyright and acknowledgements
CYP2D6 Genetic Variants

- More than 80 alleles have been identified

<table>
<thead>
<tr>
<th>Allele</th>
<th>Description</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*1</td>
<td>Wild-type</td>
<td>Regular</td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>SNPs leading to a.a. change</td>
<td>Regular</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>SNPs leading to a.a. change, unstable protein</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>SNPs leading to a.a. change</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>Splice site variants (G→A transition in intron 3/exon 4)</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>Base pair deletion in exon 5</td>
<td>Decreased</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>Gene deletion</td>
<td>None</td>
</tr>
<tr>
<td>CYP2D6*2Xn</td>
<td>Gene duplication or multiplication</td>
<td>Increased</td>
</tr>
</tbody>
</table>

Correlation between Debrisoquine Phenotype and CYP2D6 Genetic Polymorphism

- Extensive metabolizers (EMs)
- Intermediate metabolizers (IMs)
- Poor metabolizers (PMs)

Debrisoquine/4-hydroxydebrisoquine

Nortriptyline: an Excellent Example

Dalé et al. (1998) Clin Pharmacol Ther, 63, 444
**Genetics of Phase I Enzymes**

**Prof. Aiming Yu**

---

### Fluoxetine N-Demethylation by Purified CYP2D6 Allelic Isoforms

- **CYP2D6.1 Protein**
  - Predicted: 55769.6 Da
  - Measured: 55772.0 Da

---

### CYP2D6 Genetic Polymorphism: Inter-Ethnic Difference

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency of allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caucasians</td>
</tr>
<tr>
<td>CYP2D6*1</td>
<td>~36</td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>27:32</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>12:21</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>2:7</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>0.3:2</td>
</tr>
</tbody>
</table>

---

### Debrisoquine Phenotype: Inter-Ethnic Difference

- **Chinese (n=695)**
  - PMs
  - IMs

- **Swedish (n=1011)**
  - PMs
  - IMs

---

The screen versions of these slides have full details of copyright and acknowledgements.
**CYP2D6 Polymorphism: Effects on Drugs Inactivated by CYP2D6**

<table>
<thead>
<tr>
<th>Metabolizer Type</th>
<th>Time</th>
<th>Plasma Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td></td>
<td>Lack of response</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td></td>
<td>Exaggerated response</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td></td>
<td>Adverse effects</td>
</tr>
<tr>
<td>Extensive metabolizer</td>
<td></td>
<td>Desired efficacy</td>
</tr>
</tbody>
</table>

**CYP2D6 Polymorphism: Effects on Prodrugs Activated by CYP2D6**

<table>
<thead>
<tr>
<th>Metabolizer Type</th>
<th>Time</th>
<th>Plasma Metabolite Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td></td>
<td>Exaggerated response</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td></td>
<td>Diminished response</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td></td>
<td>Lack of response</td>
</tr>
<tr>
<td>Extensive metabolizer</td>
<td></td>
<td>Desired response</td>
</tr>
</tbody>
</table>

**Metabolism of Codeine**

- Codeine → T6-glucoronide
- T6-glucoronide → Morphine
- Morphine → Codeine
- Codeine → CYP3A4
- CYP2D6 → Codeine
- CYP3A4 → Codeine-6-glucoronide

The screen versions of these slides have full details of copyright and acknowledgements.
Production of Morphine from Codeine in CYP2D6 EMs and PMs

- **Cmax** (nmol/L)
- **AUC** (nmol·h/L)
- ΔEM: 173.1 ± 89.8
- CPM: 38.2 ± 15.7

170 mg codeine phosphate, p.o.

Eckhardt et al. (1998) Pain, 76, 27

Pain Tolerance in CYP2D6 EMs and PMs

- Extensive metabolizer
- Poor metabolizer
- Baseline
- Placebo
- Codeine

Eckhardt et al. (1998) Pain, 76, 27

Codeine Intoxication Associated with CYP2D6 UM

- "Small doses of codeine (25 mg three times a day, oral) was given to a patient for the treatment of a cough associated with bilateral pneumonia..."
- "On hospital day 4, the patient's level of consciousness rapidly deteriorated, and he became unresponsive... Initial neurological examination showed a score of 6 on the Glasgow Coma Scale (no eye opening, no verbal response, and limb withdrawal after pain stimulation)..."
- "Intravenous administration of naloxone (0.4 mg) that was repeated two times resulted in a dramatic improvement in the patient's level of consciousness... Two days after the acute event, the patient had recovered completely..."


The screen versions of these slides have full details of copyright and acknowledgements
CYP2D6 is Expressed in Human Brain

Substantia nigra

CYP2D6 mRNA
CYP2D6 protein

Siegle et al. (2001) Pharmacogenetics

CYP Genetic Polymorphism: Issues

- Metabolism
- Pharmacokinetics
- Pharmacodynamics
- Toxicity

CYP Genetic Polymorphism: Opportunity

- Individualized medications
  - Dose adjustment
  - Drug selection
- Prediction of a patient’s drug metabolizing capacity
  - Phenotyping
  - Use of probe drug
    (debrisoquine, dextromethorphan, sparteine for CYP2D6)
  - Genotyping
    - Allele-specific PCR, gene chips, etc.
Phenotyping towards Individualized Drug Therapy
- Identification of a probe drug
- Administration of the probe drug
- Collection of biofluid (blood/urine)
- Bioanalysis of drug and metabolite
- Estimation of metabolic capacity

Genotyping towards Individualized Drug Therapy
- Mechanisms of allelic discrimination
  - Hybridization
  - Primer extension
  - Ligase
- Reaction formats
  - Homogenous reaction
  - Solid-phase reaction (glass, silicon chip)
- Detection methods
  - Monitoring light emission (fluorescence)
  - Mass spectrometry

CYP2D6 Genotyping towards Individualized Drug Therapy
- Allele-specific PCR
  - Restriction fragment length polymorphism PCR (RFLP-PCR)
  (homogenous primer extension)
- Real-time PCR (qPCR)
  (homogenous hybridization)
- Microarray genotyping
  (hybridization on solid support with fluorescence detection)
- CYP450 genotyping test (Roche Diagnostics)
  - FDA granted approval for the tests for CYP2D6 and CYP2C19
  - The tests can be used for diagnostic purposes in both the US and EU
**Comparison between Genotyping and Phenotyping Methods**

<table>
<thead>
<tr>
<th>N</th>
<th>UM</th>
<th>IM</th>
<th>EM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>199</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Log of MR (log degradation rate of dextromethorphan over dextrorphan)


---

**Phenotyping vs. Genotyping: Pros and Cons**

**Phenotyping**
- Direct determination of true metabolic capacity
- Reliable
- Time consumption (sample collection)
- Variable due to clinic settings

**Genotyping**
- More efficient approach (high-throughput)
- Reliable
- Genotype not affected by environmental factors
- Band/signal pattern and intensity may be confusing (duplication and multiplication)
- False prediction if presence of novel alleles or compensation by other enzymes/pathways
- Drug interactions lead to inaccurate prediction

---

**Potency of CYP2D6 Inhibitor**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Ki (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>0.411</td>
</tr>
<tr>
<td>Norfluoxetine</td>
<td>1.38</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>10.1</td>
</tr>
<tr>
<td>Citalopram</td>
<td>10.9</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>55.3</td>
</tr>
<tr>
<td>Quinidine</td>
<td>0.00319</td>
</tr>
</tbody>
</table>

Yu et al. (2003) Pharmacogenetics
Drug-Drug Interactions
Altered CYP2D6 Metabolic Activity

Summary

- Genetic polymorphism of phase I CYP2D6 enzyme leads to substantial variations in psychopharmacotherapy
- Pharmacogenetics and pharmacogenomics move towards individualized healthcare
  - To achieve drug efficacy
  - To prevent adverse effects

References and Websites

- Dalma-Wiezhausz and Murphy (2003) Psychiatr Genet 12, 97
- Ingelman-Sundberg et al. (1999) Trends Pharmacol Sci 20, 342
- Wolf et al. (2000) BMJ 320, 987
- Zanger et al. (2004) Naunyn Schmiedebergs Arch Pharmacol 369, 23
- http://www.iscb.org/~p450srv/
- http://drnelson.utmem.edu/CytochromeP450.html
- http://medicine.iupui.edu/flockhart/table.htm
- http://www.imm.ki.se/CYPalleles/