Substrate-specific metabolism of CYP2D6: How can CYP2D6 phenotype prediction be improved?

Andrea Gaedigk, PhD

Children's Mercy Kansas City
Division of Clinical Pharmacology, Toxicology & Therapeutic Innovation
CYP2D6 (Debrisoquine-Sparteine Polymorphism)

- First discovered on debrisoquine and sparteine in the 1970s
- Metabolizes many clinically used drugs
- Highly polymorphic gene locus
- Large inter-individual variation of CYP2D6 activity
- Incidence of poor and ultrarapid metabolism varies widely across populations
- Risk of adverse events or treatment failure for extreme phenotypes
- Five CPIC guidelines (codeine, SSRIs, TCAs, ondansetron and tamoxifen)
Selection of drugs metabolized by CYP2D6

**Antiarrythmics**
- encainide
- flecainide
- sparteine
- perhexilene
- propafenone
- mexiletine

**β-blockers**
- Carvedilol
- S-metoprolol
- nebivolol
- Propranolone
- timolol

**Others**
- alprenolol
- amphetamine
- atomoxetine (Strattera)
- bufuralol
- chlorpheniramine
- codeine
- debrisoquine
- dexfenfluramine
- dextromethorphan
- duloxetine
- lidocaine
- metoclopramide
- methoxyamphetamine
- ondansetron
- oxycodon
- perhexilene
- phenacetin
- phenformin
- promethazine
- tamoxifene
- tramadol

**Antidepressants**
- amitriptyline
- aripiprazole
- clomipramine
- chlorpromazine
- desipramine
- duloxetine
- fluoxetine (Prozac)
- fluvoxamine
- haloperidol
- imipramine
- minaprine
- nortripyline
- paroxetine
- perphenazine
- risperidone
- thioridazine
- venlafaxine
- zuclopenthixol

**Antipsychotics**
- amitriptyline
- aripiprazole
- clomipramine
- chlorpromazine
- desipramine
- duloxetine
- fluoxetine (Prozac)
- fluvoxamine
- haloperidol
- imipramine
- minaprine
- nortripyline
- paroxetine
- perphenazine
- risperidone
- thioridazine
- venlafaxine
- zuclopenthixol

CYP2D6 activity

- Urinary metabolic ratios of a probe drug serve as a measure of CYP2D6 activity

CYP2D6 allele definitions

# of defined allelic variants: 105
# of possible diplotypes: 7752

Genetic variations comprise:
- Single nucleotide polymorphisms (SNPs)
- Small deletions or insertions (indels)
- Large deletions (e.g. entire gene)
- Gene copy number variation (CNVs)
- Structural variation (hybrid genes, tandems)

PharmVar interactive database for CYP2D6, CYP2C9 and CYP2C19 launched this week
www.PharmVar.org
Activity Score (AS)

- To facilitate translation of diplotypes into phenotype
  - Assigning a value to each allele reflecting its perceived activity towards a given substrate
  - \( \text{AS} = \text{sum of the values of both alleles} \)
  - Duplicated genes receive double the value of their single counterparts
  - Used in CPIC guidelines

<table>
<thead>
<tr>
<th>Functional Status</th>
<th>Value assigned</th>
<th>Alleles (selection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased function</td>
<td>2 (3)</td>
<td>*1x2, *2x2, *35x2, *45x2 (x3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*9x2, *10x2, *17x2, *29x2.....</td>
</tr>
<tr>
<td>Decreased function</td>
<td>0.5</td>
<td>*9, *10, *14B, *17, *29, *41, *49, etc</td>
</tr>
</tbody>
</table>
### Activity Score (AS)

<table>
<thead>
<tr>
<th>Metabolizer Status</th>
<th>Activity Score</th>
<th>Diplotypes (selection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer (UM)</td>
<td>≥2.5</td>
<td>*1/*2x2, *2x2/*35x2…</td>
</tr>
<tr>
<td>Normal metabolizer (NM)</td>
<td>1.5-2</td>
<td>*1/*1, *1/*2, *1/*41, *2/*10…</td>
</tr>
<tr>
<td>Poor metabolizers (PM)</td>
<td>0</td>
<td>*4/*5, *3/*6, *12/*84…</td>
</tr>
</tbody>
</table>

**AS = 1**

**AS = 0.5**
Relationship between DM/DX and AS

Subjects: 672
Alleles tested: 21 + gene duplications
Genotypes: 94
Activity Score groups: 6
Variability explained by AS: 55%
Variability explained by genotype: 59%

Limitations and challenges of the Activity Score

- Accuracy of the function/activity assigned to an allele
  - Categorical (no, decreased, normal and increased function)
  - No or limited data

- Different ways to measure “activity” or “metabolic capacity”
  - What is the best experimental approach to determine the activity of an allele or diplotype?

- Activity of an allele may be substrate-specific
  - Substrate may act as inhibitor
Genotype-based dose adaptations as % of recommended ‘usual’ dose

PGx of antidepressants and antipsychotics: contribution of allelic variation to the phenotype of drug response – a meta analysis
CYP2D6*17: wide range of activity

<table>
<thead>
<tr>
<th>Substrate</th>
<th>CYP2D6.17 % of CYP2D6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>chomipramine</td>
<td>15</td>
</tr>
<tr>
<td><strong>dextromethorphan</strong></td>
<td><strong>29</strong></td>
</tr>
<tr>
<td>fluphenazine</td>
<td>34</td>
</tr>
<tr>
<td>bufuralol</td>
<td>38</td>
</tr>
<tr>
<td>timolol</td>
<td>46</td>
</tr>
<tr>
<td>metoprolol</td>
<td>58</td>
</tr>
<tr>
<td>propafenone</td>
<td>62</td>
</tr>
<tr>
<td>thioridazine</td>
<td>70</td>
</tr>
<tr>
<td><strong>debrisoquine</strong></td>
<td><strong>71</strong></td>
</tr>
<tr>
<td>sparteine</td>
<td>87</td>
</tr>
</tbody>
</table>

In-vitro systems

### COS-7 cell expressed protein

<table>
<thead>
<tr>
<th>Protein</th>
<th>DM $V_{max}/K_m$</th>
<th>Bufuralol $V_{max}/K_m$</th>
<th>% CYP2D6.1</th>
<th>% CYP2D6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6.2</td>
<td>71</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6.17</td>
<td>25</td>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


### Baculovirus expressed protein (Supersomes)

<table>
<thead>
<tr>
<th>Protein</th>
<th>DM $V_{max}/K_m$</th>
<th>Bufuralol $V_{max}/K_m$</th>
<th>Debrisoquine $V_{max}/K_m$</th>
<th>% CYP2D6.1</th>
<th>% CYP2D6.1</th>
<th>% CYP2D6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6.2</td>
<td>109</td>
<td>117</td>
<td>101</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6.17</td>
<td>18</td>
<td>22</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### In-vitro systems

<table>
<thead>
<tr>
<th>substrate</th>
<th>CYP2D6.10 % of CYP2D6.1</th>
<th>CYP2D6.17 % of CYP2D6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>nortriptyline</td>
<td>1.3</td>
<td>7.3</td>
</tr>
<tr>
<td>bufuralol</td>
<td>3.7</td>
<td>22.8</td>
</tr>
<tr>
<td>dextromethorphan</td>
<td>5.3</td>
<td>16.8</td>
</tr>
<tr>
<td>tramadol</td>
<td>6.9</td>
<td>35.7</td>
</tr>
<tr>
<td>fluoxetine</td>
<td>7.5</td>
<td>8.17</td>
</tr>
<tr>
<td>atomoxetine</td>
<td>8.6</td>
<td>21.9</td>
</tr>
<tr>
<td>debrisoquine</td>
<td>11.8</td>
<td>64.2</td>
</tr>
<tr>
<td>codeine</td>
<td>27.9</td>
<td>80.4</td>
</tr>
</tbody>
</table>

Baculovirus-expressed protein (Supersomes)
CYP2D6 activity in human liver

- Protein level is a better predictor than AS
- Protein levels not an option to test patients
- Difficult to find tissues with genotypes of interest

Ning et al (2018) CPT Epub ahead of print
36 male subjects
Plasma and urine
Data modeled simultaneously using the population pharmacokinetics NONMEM software
Five-compartment model adequately described the data
Urinary pH was confirmed as a significant covariate for DM renal clearance
~55% of variability explained by genotype

Values of 1, 0.4 and 0.17 should be used for *1, *2 and *41, respectively
No data for other alleles
Many alleles are rare – difficult to find subjects
Substrate specificity - tamoxifen

Plasma metabolic ratio of endoxifen/N-desmethyl-tamoxifen
How can the Activity Score system be improved?

- How should function be determined to best inform the translation of genotype to phenotype?
  - A standardized approach to assign function is needed
  - Function currently assigned based on varied knowledge/evidence
  - A single activity label does likely not fit all substrates
  - Need a better understanding of other factors impacting metabolic capacity of an individual
Genotype translation standardization project

- **CPIC expert working group**
  Find consensus of how to translate genotype into phenotype groups

Tweak the system by ‘downgrading’ CYP2D6*10, a severely decreased activity allele and introduce a value of 0.25 for Activity Score assignments
Percent Activity (PA) system

- Conceived from the discussions of the CPIC working group
  - System proposed by Daniel Hertz
  - Average of 2 alleles on a 0-1 scale vs adding 2 alleles on a 0-2 scale
  - Minimize information loss during allelic activity and phenotype assignment
  - More flexible, precise and intuitive meaning, e.g. 80%
  - Translate PA% into phenotype
  - Reanalyze existing large datasets (e.g. endoxifen/N-desmethyl-tamoxifen ratios as proof of concept)
GOLDILOKS

Genomic- and Ontogeny- Linked Dose Individualization and Clinical Optimization for Kids

"Not too big, not too small ... the dose of medication that is 'just right' for your child"
U54 translational study

- Enhancer SNP 116 kb downstream of gene locus
- Increases transcription levels
- How much of the variability does the enhancer SNP explain?
- Which alleles have the enhancer SNP?
- Clinically relevant?
- Need to incorporate into the AS?

U54 clinical study

- Improve current PBPK model for atomoxetine
  - Published dataset (n=24); Validation study (n=24) nearly completed
  - Does the enhancer SNP improve phenotype prediction?

- Clinical Study
  - 120 subjects over 2 years (first patients enrolled)
  - Extensive clinical testing for ADHD diagnosis and response
  - Patients dosed to target exposure based on CYP2D6 genotype
  - PK study on 2 occasions
  - Genotyped for other PGx genes that may affect PK and PD

- Refine model

- Metabolomics
  - to identify biomarker predictive of response
Atomoxetine-specific dosing algorithm

What a GOLDILOKs atomoxetine interface might look like to guide precision drug therapy
Acknowledgements

Division of Clinical Pharmacology at Children’s Mercy
- Steven Leeder
- Susan Abdel-Rahman
- Jean Dinh
- Leon Van Haandel
- Robin Pearce
- Chengpeng “Charlie” Bi
- Vincent Staggs
- Carla Allan
- Jaylene Weigel
- Technical support staff

Center for Pediatric Genomics at CMH
- Sarah Soden
- Neil Miller
- Greyson Twist
- Scott Casey
- Emily Farrow

FUNDING
GOLDILOKS: U54 HD090258-01
PharmVar: R24 GM123930-01