Therapeutic Drug Monitoring and Pharmacogenetics

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Learning Objectives

• Describe the general guidelines for TDM testing
• Discuss the basic components of pharmacokinetics and pharmacogenetics
• Identify commonly monitored drugs
• List important consideration for TDM methods
• Explain barriers to implementation of PGx testing
• Recognize clinical areas where implementation of TDM or PGx may have a positive impact on patient care
Definition of TDM

• **a priori TDM**
  – pharmacogenetic information
  – demographic information
  – clinical information

• **a posteriori TDM**
  – Pharmacokinetic monitoring
  – Pharmacodynamic monitoring
General Criteria for TDM

• Narrow therapeutic index
• Defined therapeutic range and toxic threshold
• Good relationship between [blood] and clinical/toxic effect
• Poor relationship between drug dose and [blood]
• Significant inter-individual variation
• Serious consequences for under- or over-dosing
• Subject to drug-drug interactions
• Knowledge of the drug level influences management
• When toxicity mimics indication for which drug is prescribed
Therapeutic Window

- Hypodosage
- Therapeutic Window
- Hyperdosage
- Overdose/Death

--- Increasing Response

--- Increasing Dosage
Steady-state and therapeutic index
ED50 = the dose of drug in which 50% of treated individuals will experience benefit
TD50 = the dose of drug in which 50% of individuals will experience toxic adverse effects
LD50 = the dose of drug in which 50% of individuals will result in morbidity

Image from pharmacologycorner.com
### Factors that influence TDM Results

<table>
<thead>
<tr>
<th>Drug</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Route of administration</td>
</tr>
<tr>
<td></td>
<td>Dose regimen</td>
</tr>
<tr>
<td></td>
<td>Pharmacokinetics (Vd, half-life, metabolites)</td>
</tr>
<tr>
<td>Patient</td>
<td>Age (pediatric, geriatric)</td>
</tr>
<tr>
<td></td>
<td>Body composition</td>
</tr>
<tr>
<td></td>
<td>Renal function</td>
</tr>
<tr>
<td></td>
<td>Hepatic function</td>
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<tr>
<td></td>
<td>Compliance</td>
</tr>
<tr>
<td></td>
<td>Pregnancy</td>
</tr>
<tr>
<td></td>
<td>Protein status</td>
</tr>
<tr>
<td></td>
<td>Pharmacogenetics</td>
</tr>
<tr>
<td></td>
<td>Disease / Malignancies</td>
</tr>
</tbody>
</table>
Factors that influence TDM Results

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Collection tube, preservatives</th>
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</thead>
<tbody>
<tr>
<td>Time collected relative to dose</td>
<td></td>
</tr>
<tr>
<td>Sampling methods</td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td></td>
</tr>
<tr>
<td>Handling</td>
<td></td>
</tr>
<tr>
<td>Analytical method</td>
<td>Preanalytical processing (extraction)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
</tr>
<tr>
<td>Matrix effects</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Concominant medications</td>
</tr>
<tr>
<td>Supplements</td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td></td>
</tr>
<tr>
<td>Clerical errors</td>
<td></td>
</tr>
</tbody>
</table>
Factors that influence TDM Results

Different Routes of Administration

- I - fast-dissolving tablet
- II - slower dissolving tablet
- III - sustained-release tablet
- IV - tablet with poor bioavailability

Different Liberation processes

Clinical Chemistry: Theory, Analysis, Correlation, 5th ed.
Factors that influence TDM Results

Influence of Metabolism Process

- Enzyme inhibition
- Enzyme induction
- Normal

Influence of Elimination Process

- Infusion
- Renal failure
- Normal

Clinical Chemistry: Theory, Analysis, Correlation, 5th ed.
General Guidelines for TDM

- Preferred specimen is serum or plasma at steady state
  - Whole blood is needed for immunosuppressant monitoring
  - For some tests, plasma is not acceptable due to interferences from anticoagulant

- Trough levels are collected shortly before the next dose

- TDM is indicated after changes in the dose or timing of administration

- It is critical to wait until to do testing until a new equilibrium has been established after a change in dosing

- TDM is indicated when patient is experiencing signs and symptoms that suggest therapeutic failure or toxicity

- Specific guidelines depend on the drug, the approach to drug delivery, the clinical scenario and the needs of the patient
General Guidelines for TDM

• Establish baseline concentrations whenever possible

• Evaluate potential causes for lack of efficacy
  • Differential metabolizers (fast, slow, altered)
  • Noncompliance
  • Drug-drug interactions

• Evaluate potential causes for toxicity
  • Altered drug utilization due to physiological conditions (adolescence, geriatrics)
  • Altered drug utilization due to pathological conditions (renal or liver failure)
  • Differential metabolizers (fast, slow, altered)
  • Drug-drug interactions
## Testing Methodologies

<table>
<thead>
<tr>
<th>Testing Methods</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point-of-care assays</td>
<td>POC</td>
</tr>
<tr>
<td>Radio immunoassay</td>
<td>RIA</td>
</tr>
<tr>
<td>Enzyme linked immunosorbent assay</td>
<td>ELISA</td>
</tr>
<tr>
<td>Enzyme-multiplied immunoassay technique</td>
<td>EMIT</td>
</tr>
<tr>
<td>Cloned enzyme donor immunoassay</td>
<td>CEDIA</td>
</tr>
<tr>
<td>Fluorescence polarization immunoassay</td>
<td>FPIA</td>
</tr>
<tr>
<td>Liquid chromatography with ultraviolet detection</td>
<td>HPLC-UV</td>
</tr>
<tr>
<td>Gas chromatography mass spectrometry</td>
<td>GC-MS</td>
</tr>
<tr>
<td>Liquid chromatography tandem mass spectrometry</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>Liquid chromatography time-of-flight mass spectrometry</td>
<td>LC-TOF</td>
</tr>
<tr>
<td>Liquid chromatography high resolution mass spectrometry</td>
<td>LC-HRMS</td>
</tr>
</tbody>
</table>
## Assay Standardization - AMR

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sample Prep</th>
<th>IS</th>
<th>Column</th>
<th>Detection</th>
<th>Run</th>
<th>AMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPA 1</td>
<td>PPT – 100ul S</td>
<td>MPAC</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>4</td>
<td>0.1-50 ug/mL</td>
</tr>
<tr>
<td>MPA 2</td>
<td>PPT – 100ul P</td>
<td>Indomethacine</td>
<td>C18</td>
<td>ESI-QQQ -</td>
<td>6</td>
<td>0.1-30 ug/mL</td>
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<tr>
<td>MPA 3</td>
<td>SPE – 50ul S</td>
<td>MPAC</td>
<td>dC18</td>
<td>ESI-QQQ +</td>
<td>7</td>
<td>0.1-16 ug/mL</td>
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<tr>
<td>MPA 4</td>
<td>Online – 50ul P</td>
<td>Cyclosporin D</td>
<td>POROS</td>
<td>ESI-QQQ +</td>
<td>5</td>
<td>0.05-50 ug/mL</td>
</tr>
<tr>
<td>CsA 1</td>
<td>PPT – 100ul WB</td>
<td>PSC833</td>
<td>C8</td>
<td>APCI-Q +</td>
<td>10</td>
<td>1-2,500 ng/mL</td>
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<tr>
<td>CsA 2</td>
<td>PPT/SPE – 50ul WB</td>
<td>Cyclosporin D12</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>2</td>
<td>10-2,000 ng/mL</td>
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<tr>
<td>CsA 3</td>
<td>Online – 50ul P</td>
<td>Cyclosporin C</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>3</td>
<td>1-4,000 ng/mL</td>
</tr>
<tr>
<td>CsA 4</td>
<td>SPE – 250ul WB</td>
<td>Cyclosporin D</td>
<td>hypersil</td>
<td>ESI-Q +</td>
<td>6</td>
<td>N/A</td>
</tr>
<tr>
<td>Tacro 1</td>
<td>LLE – 250ul WB</td>
<td>FR298701</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>10</td>
<td>0.2-20 ng/mL</td>
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<tr>
<td>Tacro 2</td>
<td>Online – 500ul WB</td>
<td>Ascomycin</td>
<td>C18</td>
<td>ESI-Q +</td>
<td>3</td>
<td>1-80 ng/mL</td>
</tr>
<tr>
<td>Tacro 3</td>
<td>PPT – 80ul WB</td>
<td>Ascomycin</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>2.5</td>
<td>0.52-155 ng/mL</td>
</tr>
<tr>
<td>Evero 1</td>
<td>PPT – 100ul WB</td>
<td>Hydroxy-propylrapamycin</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>2</td>
<td>0.5-40 ng/mL</td>
</tr>
<tr>
<td>Evero 2</td>
<td>PPT-Online WB</td>
<td>Ascomycin</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>2.8</td>
<td>1-50 ng/mL</td>
</tr>
<tr>
<td>Siro 1</td>
<td>Online – 500ul WB</td>
<td>Ascromycin</td>
<td>C18</td>
<td>ESI-Q +</td>
<td>3</td>
<td>1-80 ng/mL</td>
</tr>
<tr>
<td>Siro 2</td>
<td>PPT – 80ul WB</td>
<td>Desmethoxyrapamycin</td>
<td>C18</td>
<td>ESI-QQQ +</td>
<td>2.5</td>
<td>0.47-94.8 ng/mL</td>
</tr>
</tbody>
</table>
International PT Scheme

Tacrolimus (5-15 ng/mL)
- CEDIA (n = 11)
- Architect (n = 152)
- ACMA (n = 89)
- Others (n = 5)
- EMIT (n = 38)
- HPLC/MS (n = 129)

Sirolimus (4-12 ng/mL)
- Other (n = 16)
- Abbott Architect (n = 55)
- Abbott Imx (n = 1)
- HPLC/MS (n = 106)
- HPLC/UV (n = 3)

Everolimus (3-8 ng/mL)
- All Other Methods (n = 16)
- Innofluor (n = 38)
- HPLC (n = 89)

MPA (1-3.5 ng/mL)
- Others (n = 21)
- HPLC / MS (n = 39)
- EMIT (n = 53)
- HPLC / UV (n = 52)
Pharmacokinetics: CLADME

- Compliance: is the patient taking the drug
- Liberation: release of the drug from the pharmaceutical preparation
- Absorption
- Distribution
- Metabolism
- Excretion
ADME - Absorption

“The transfer of a drug or other xenobiotic from its site of administration to the bloodstream”

• Drug Partitioning
  • drugs can be characterized by “partition coefficients”
    • ratio of solubility in an aqueous, polar solvent vs. a lipophilic, non-polar solvent
    • lipophilic drugs are rapidly absorbed
  • variables:
    • body composition
    • pH (blood and urine)
    • ionization – function of pKa (markedly reduces lipophilicity)
ADME - Absorption – Drug Transport

- Passive diffusion - transport driven by conc. gradient (95% of all drugs)
- Active transport – transport against the conc. gradient requires energy, can be receptor mediated
- Facilitated transport – follows the conc. gradient, requires energy, can be receptor mediated
- Convection transport – transport through water filled pores
- Pinocytosis – cell engulfs the drug
ADME - Distribution

“Movement of a drug or xenobiotic from the bloodstream to the site of action”

1) Drug remains in blood
2) Drug enters extravascular fluids
3) Drug migrates into various tissues/organs

Protein Binding – reduces the volume of distribution

• acidic drugs – albumin
• basic drugs – alpha 1 – acid glycoprotein and lipoproteins
• free drug is sometimes measured - bound drug >90
• free drug is the biologically active form of the drug
ADME - Distribution

\[ \text{Vd} = \frac{\text{dose}}{[\text{plasma}]} \]

\[ \text{Vd} > 3\text{L (outside plasma)} - \text{plasma volume of avg. adult } \sim 3\text{L} \]

Limitations:
- does not estimate actual sites of distribution
- does not account for individual differences
- requires drug distribution to be complete = \( C_{ss} \)

Applications of Vd:
1) Loading dose = \( \text{Vd} \times [\text{drug}]_{ss} \)
2) Dose adjustments = \( \text{Vd} ([\text{drug}]_{\text{desired}} - [\text{drug}]_{\text{initial}}) \)
ADME - Metabolism

Phase I – Oxidation, Reduction, Methylation, Hydroxylation, Deamination
Phase 2 – Conjugation (D-glucuronidation, O-sulfation, N-acetylation, O-, N-, S-methylation, glutathione, amino acid conjugation)

William E. Evans* and Mary V. Relling SCIENCE VOL 286 15 OCTOBER 1999
Genetic Polymorphisms: Drug Concentration and Drug Effect

**Drug Metabolism Genotypes**

**Drug Receptor Genotypes**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Effect (%)</th>
<th>Therapeutic Effect (%)</th>
<th>Toxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt/wt</td>
<td>75</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>wt/m</td>
<td>35</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>m/m</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Drug Concentration vs. Time**

**Effect (%) vs. Drug Concentration**

**Time**

**Drug Concentration**

A. wt/wt

B. wt/m

C. m/m

William E. Evans* and Mary V. Relling

SCIENCE VOL 286 15 OCTOBER 1999
Treatment Modifications and Patient Genotypes

Genotype: WT/WT, Variant/WT, V/V

Drug Metabolism: Good, Intermediate, Poor

Drug Receptor: Good, Intermediate, Poor

Metabolism: Good, Intermediate, Poor

Receptor Sensitivity: Good, Intermediate, Poor

No change in dose or drug

Dose

Dose

Dose

Alternative drug
ADME - Excretion/Elimination

Primary Organs – liver, lungs and kidneys

Sum of clearance by all body pathways:
\[ C_{\text{total}} = C_{\text{renal}} + C_{\text{hepatic}} + C_{\text{pulmonary}} + \ldots \]

Factors that influence Excretion:
1) BMI
2) Cardiac output (bloodflow)
3) Hepatic and renal fx
4) Protein status
ADME - Excretion/Elimination

Kinetics

First-order kinetics – rate of elimination is proportional to the amount of drug present

Zero-order kinetics – rate of elimination is constant regardless of the amount of drug present in the system (ethanol, phenytoin, salicylates)

Capacity-limited kinetics – occurs when the rate of elimination shifts from first-order to zero-order based on the saturation of the elimination processes (overdoses)

<table>
<thead>
<tr>
<th>Reaction Order</th>
<th>[ ] vs. time plot</th>
<th>Rate of Reaction</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>Linear</td>
<td>Constant</td>
<td>Proportional to [ ]</td>
</tr>
<tr>
<td>First</td>
<td>Exponential</td>
<td>Proportional to [ ]</td>
<td>Constant</td>
</tr>
</tbody>
</table>
Commonly Monitored Drugs

- **Cardioactive drugs**: digoxin, procainamide
- **Antiepileptic drugs**: valproic acid, phenobarbital, phenytoin, carbamazepine
- **Antibiotics**: amikacin, gentamicin, vancomycin, tobramycin
- **Immunosuppressants**: cyclosporine, tacrolimus, sirolimus
- **Antidepressants**: nortriptyline, desipramine, lithium
- **Bronchodilators**: theophylline
- **Others**: methotrexate, busulfan, antifungal, HIV, antipsychotic
Antiepileptic Drugs (AEDs)

- AEDs – valproic acid, phenytoin, carbamezepine, phenobarbital
- Newer AEDs (lamotrigine, gabapentin, topiramate, levetiracetam, oxcarbazepine) are not widely monitored
- There is a defined relationship between blood concentration and seizure control
- Large individual differences between dose and blood level
- CYP450 metabolized, patients on multiple drugs
- Both under-dosing and over-dosing can result in seizures
Antibiotics

• Most antibiotics (B-lactams, macrolides, quinolones) have a wide therapeutic index and do not require monitoring
• Aminoglycosides (gentamicin, amikacin, streptomycin and tobramycin) and vancomycin have a narrow therapeutic index and toxicity may be severe or irreversible (nephrotoxic)
• Aminoglycoside kinetics display great variation dependent on disease state
• Infections are associated with altered hydration and membrane permeability
Ordering Trends at SFGH

Number of Orders

- Digoxin
- Valproic Acid
- Phenytoin (DPH)
- Phenobarbital
- Carbamezepine
- Gentamicin
- Vancomycin
- Tobramycin
Ordering Trends at SFGH

Number of Orders

- Clozapine
- Cyclosporine A
- Gabapentin
- Haloperidol
- Keppra
- Lamotrigine
- Tacrolimus

Years: 2006 to 2012
Pharmacogenetics
Top 10 PGx tests in clinical laboratories (2005)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>2</td>
<td>TPMT (thiopurine S-methyltransferase)</td>
</tr>
<tr>
<td>3</td>
<td>CYP2C9</td>
</tr>
<tr>
<td>4</td>
<td>CYP2C19</td>
</tr>
<tr>
<td>5</td>
<td>NAT-2 (N-acetyl transferase)</td>
</tr>
<tr>
<td>6</td>
<td>CYP3A5</td>
</tr>
<tr>
<td>7</td>
<td>UGT1A1 (Uridinediphosphoglucuronosyltransferase)</td>
</tr>
<tr>
<td>8</td>
<td>MDR1/P-Glycoprotein (medium-chain dehydrogenase/reductase)</td>
</tr>
<tr>
<td>9</td>
<td>CYP2B6</td>
</tr>
<tr>
<td>10</td>
<td>MTHFR (Methylene tetrahydrofolate reductase)</td>
</tr>
</tbody>
</table>

Do You Offer/Plan To Offer CYP450 or UGT1A1 Testing?

Source: Washington G-2 Reports' 2006 Molecular Diagnostic Test Survey
# Specificity of Common PGx Biomarkers

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene target (Ref.)</th>
<th>ADR</th>
<th>Odds ratio</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>HLA-B*1502</td>
<td>Stevens–Johnson syndrome (Asian)</td>
<td>1023</td>
<td>[46]</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>HLA-B*5801</td>
<td>Stevens–Johnson syndrome</td>
<td>580</td>
<td>[27]</td>
</tr>
<tr>
<td>6-mercaptopurine</td>
<td>TMPT</td>
<td>Neutropenia</td>
<td>49</td>
<td>[37]</td>
</tr>
<tr>
<td>Abacavir</td>
<td>HLA-B*5701</td>
<td>Hypersensitivity</td>
<td>33</td>
<td>[15]</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>TNF-α -238G/A</td>
<td>Cholestatic hepatitis</td>
<td>31</td>
<td>[47]</td>
</tr>
<tr>
<td>Irinotecan (high doses)</td>
<td>UGT1A1*28</td>
<td>Severe neutropenia</td>
<td>8–28</td>
<td>[48,49]</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>HLA-DRB1*0101</td>
<td>Hypersensitivity</td>
<td>18</td>
<td>[50]</td>
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<tr>
<td>Flucloxacillin</td>
<td>HLA-DRB1-DQB1</td>
<td>Cholestatic hepatitis</td>
<td>17</td>
<td>[47]</td>
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<tr>
<td>Simvastatin</td>
<td>SLC01B1*5</td>
<td>Myopathy</td>
<td>17</td>
<td>[38]</td>
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<tr>
<td>Nevirapine</td>
<td>HLA Cw8-B14</td>
<td>Hypersensitivity</td>
<td>15</td>
<td>[51]</td>
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<tr>
<td>Warfarin</td>
<td>VKORC1 and CYP2C9</td>
<td>Oral anticoagulant overdose</td>
<td>10</td>
<td>[52]</td>
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<tr>
<td>NSAID</td>
<td>GSTM1 and GSTT1</td>
<td>Cytolytic hepatitis</td>
<td>9</td>
<td>[53]</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>UGT2B7*2</td>
<td>Hepatotoxicity</td>
<td>8</td>
<td>[54]</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>ABCC2 C-24T</td>
<td>Hepatotoxicity</td>
<td>5</td>
<td>[54]</td>
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<tr>
<td>Isoniazid</td>
<td>NAT2</td>
<td>Cytolytic hepatitis</td>
<td>4–5</td>
<td>[55,56]</td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>HLA-DRB1*0701</td>
<td>Cytolytic hepatitis</td>
<td>4</td>
<td>[57]</td>
</tr>
<tr>
<td>Tacrine</td>
<td>GSTM1 and GSTT1</td>
<td>Cytolytic hepatitis</td>
<td>3</td>
<td>[58]</td>
</tr>
</tbody>
</table>

ADR: Adverse drug reaction; NSAID: Nonsteroidal anti-inflammatory drug.
PGx Case 1 and 2: Warfarin

- 40-year-old female admitted for massive pulmonary thromboembolism underwent anticoagulant and fibrinolytic therapy, following which warfarin was needed in unusually high doses to achieve effective anticoagulation
  - CYP2C9 – c.430CC and c.1075AA (rapid warfarin metabolism)
  - VKORC1 – c.-1639GG variant (low sensitivity to warfarin)

- 76-year-old male with permanent atrial fibrillation developed excessive prolongation of prothrombin time after being treated with 5 mg/day warfarin for 5 days
  - CYP2C9 – c430CC and c.1075AC
  - VKORC1 – 1639AA
  - High sensitivity to warfarin

Cortez-Dais N et al. Rev Port Cardiol 28(9):995-1004, 2009
Warfarin Pharmacogenomics
Genetic factors alone account for ~56% variability in dosing

**Warfarin Facts:**

- Most widely prescribed anticoagulant
- 3 million patients in US
- Accounts for 58,000 ER visits a year
- Top 10 drugs - # of ADRs
- Complicated dosing strategy
- Monitored by INR
- Genetic testing could save ~1 billion
- Currently – multiple dosing algorithms
• *2/*3: low dose
• *1: high dose
• A: low dose
• G: high dose

Required Patient Information

- Age:
- Sex: [Select]
- Ethnicity: [Select]
- Race: [Select]
- Weight: [ ] lbs or [ ] kgs
- Height: [ ] feet and [ ] inches or [ ] cms
- Smokes: [Select]
- Liver Disease: [Select]
- Indication: [Select]
- Baseline INR: [ ]
- Target INR: [ ]
- Randomize & Blind: [ ]
- Amiodarone/Cordarone® Dose: [ ] mg/day
- Statin/HMG CoA Reductase Inhibitor: [Select]
- Anyazole (e.g., Fluconazole): [Select]
- Sulfamethoxazole-Septra/Bactrim/Cotrim/Sulfatrim: [Select]

Genetic Information

- VKORC1-1639/3673: Not available/pending
- CYP4F2 V433M: Not available/pending
- GGCX rs11676382: Not available/pending
- CYP2C9*2: Not available/pending
- CYP2C9*3: Not available/pending
- CYP2C9*5: Not available/pending
- CYP2C9*6: Not available/pending

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ESTIMATE WARFARIN DOSE
PGx Case 3: Tamoxifen

• 45 year old premenopausal female
• PMH – major depressive disorder for 10 years, treated successfully for 12 months with fluoxetine 20 mg daily followed by 8-9 years free of symptoms
• Diagnosed with estrogen receptor (ER) positive invasive breast cancer, underwent treatment with surgery, chemotherapy and radiation therapy
• Treated with tamoxifen (SERM) to decrease likelihood of recurrence – past 6 months
• Tamoxifen well tolerated except moderate hot flashes
• She developed recurrent depressive symptoms and sought treatment from her psychiatrist
• What pharmacological agents are options to treat her depression without compromising tamoxifen efficacy

Tamoxifen Pharmacogenomics

- Tamoxifen is a SERM
- Used to treat breast cancer
- Competitively binds to the ERs
- Tamoxifen is a prodrug
- $4$-hydroxy/END = 30-100X potent

The CYP2D6*4/*4 genotype is associated with poorer relapse-free time and disease free survival in women on tamoxifen adjuvant therapy.
Tamoxifen Pharmacogenomics

END level vs CYP2D6 genotype

CYP2D6 PM Genotype = Low Endoxifen Levels

Low Endoxifen Levels ≠ CYP2D6 PM Genotype

Sertraline, citalopram, celecoxib, diphenhydramine, chlorpheniramine

SSRIs, paroxetine and fluoxetine used to relieve hot flashes

CYP2D6 PM Genotype = Low Endoxifen Levels
Low Endoxifen Levels ≠ CYP2D6 PM Genotype
PGx Case 4: Opiate Metabolism


Day 1 – full-term healthy male infant delivered, mother on 30 mg codeine/500 mg APAP for pain
Day 7 – difficulty breastfeeding and lethargy
Day 11 – well-baby visit, baby had regained birthweight
Day 12 – grey skin and milk intake decreased
Day 13 – infant found dead
Postmortem – morphine blood concentration = 70 ng/mL
(normal in neonates breastfed by mothers on codeine 0-2.2 ng/mL)

Genotype analysis of CYP2D6 – mother heterozygous for CYP2D6*2A allele with CYP2D6*2x2 gene duplication – ultra-rapid metabolizer

Neonates invariably have impaired capacity to metabolize and eliminate morphine
PGx Case 5: Adverse Drug Reaction?

- 47 year old female with a history of severe chronic depression presented to primary care doctor with rash
- She was given Solu-Medrol and Toradol which did not help
- 3 days later she admitted to the hospital (Enlow Medical Center) with a generalized rash (no bullae or conjunctival involvement), fever, flu-like symptoms (sepsis – group A strep)
- Medications discontinued– Wellbutrin, Depakote, Trazodone, Klonopin, Cymbalta, Lamotrigine (3 weeks)
- HD2 – SOB, hypertension, bullous lesions on anterior and posterior part of her chest and ulcerations in her mouth, difficulty swallowing
- Due to deteriorating condition patient was transferred to UC-Davis and admitted to the Burn Unit
- She was intubated, received an ND-tube for nutrition, placed on propofol drip for sedation, her wounds were treated with Biobrane
- Sluffing of her mucous membranes – 24% involvement
Lamotrigine-Induced Severe Cutaneous Reaction

Medications commonly implicated:
- anti-gout agents
- Antibiotics
- antipsychotics
- antiepileptics
- analgesics
- NSAIDS
Severe Cutaneous Adverse Reactions

<table>
<thead>
<tr>
<th></th>
<th>SJS</th>
<th>TEN</th>
<th>‘Hypersensitivity’/DRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucous membrane</td>
<td>&gt;90%</td>
<td>&gt; 90%</td>
<td>&lt;30%</td>
</tr>
<tr>
<td>Erosions</td>
<td>Several sites</td>
<td>Several sites</td>
<td>Mouth and lips</td>
</tr>
<tr>
<td>Detachment of epidermis</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>&lt;10% of BSA</td>
<td>&gt;30% of BSA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>Hyperkeratosis/desquamation</td>
<td>No</td>
<td>No</td>
<td>Usual</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>No</td>
<td>30%</td>
<td>No</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>No</td>
<td>No</td>
<td>90%</td>
</tr>
<tr>
<td>Atypical lymphocytes</td>
<td>No</td>
<td>No</td>
<td>30–40%</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>Bronchial erosions/ARDS</td>
<td>Bronchial erosions/ARDS</td>
<td>Interstitial pneumonitis</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatitis 10%</td>
<td>Hepatitis 10%</td>
<td>Hepatitis 60%</td>
</tr>
<tr>
<td>Heart</td>
<td>No</td>
<td>No</td>
<td>Myocarditis</td>
</tr>
<tr>
<td>Lymph node enlarged</td>
<td>No</td>
<td>No</td>
<td>Usual</td>
</tr>
</tbody>
</table>

ARDS, Adult respiratory distress syndrome; BSA, body surface area; DRESS, drug reaction with eosinophilia and systemic symptoms; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

<sup>a</sup> When detachment involves 10–29% of BSA, we classify the case as SJS–TEN overlap.

Reproduced from Roujeau and Stern [2], with permission.
### Table 2: Association between HLA allele/related loci and drug hypersensitivity is drug specific, phenotype specific and specific for population

<table>
<thead>
<tr>
<th>Culprit drug</th>
<th>Drug hypersensitivity</th>
<th>HLA association</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>SJS/TEN</td>
<td>HLA-B*1502 (corrected $P = 3.1 \times 10^{-27} - 1.6 \times 10^{-41}$, OR 1357–2504)</td>
<td>Han Chinese in Taiwan</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>SJS</td>
<td>Weak association with B44. No association with HLA-B*1502.</td>
<td>Whites in Europe</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>SJS/TEN</td>
<td>HLA-B*1502</td>
<td>South-eastern Asians</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>HSS</td>
<td>rs2894342 of motilin gene in the MHC region (corrected $P = 0.0064$, OR 7.1)</td>
<td>Han Chinese in Taiwan</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>MPE</td>
<td>HLA-A*3101 (corrected $P = 2.2 \times 10^{-3}$, OR 17.5)</td>
<td>Han Chinese in Taiwan</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Severe hypersensitivity reactions (HSS/MPE)</td>
<td>TNF2-DR3-DQ2 haplotypes ($P=0.02$, OR 3.2)</td>
<td>Whites in Europe</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>SJS/TEN/HSS</td>
<td>HLA-B*5801 (corrected $P = 4.7 \times 10^{-24}$, OR 580.3)</td>
<td>Han Chinese in Taiwan</td>
</tr>
<tr>
<td>Abacavir</td>
<td>Drug hypersensitivity (HSS/MPE)</td>
<td>HLA-B*5701 (corrected $P = 5.2 \times 10^{-20}$, OR 960)</td>
<td>Whites</td>
</tr>
<tr>
<td>Abacavir</td>
<td>Drug hypersensitivity (HSS/MPE)</td>
<td>No association with HLA-B*5701</td>
<td>Hispanics, or African</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>Drug hypersensitivity</td>
<td>HLA-DRB1*0101</td>
<td>Whites in Australia</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>Drug hypersensitivity</td>
<td>HLA-Cw8-B14 haplotype (corrected $P=0.05$)</td>
<td>Whites in Italy</td>
</tr>
</tbody>
</table>

HLA-B*5701 test orders by Qr 2002-2008
PCR-SSOP (sequence specific oligonucleotide probes)

One Lambda LABType® SSOP HLA-B
Luminex® XMAP® technology
<table>
<thead>
<tr>
<th>Study</th>
<th>B*5701 positive</th>
<th>B*5701 negative</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sanchez-Giron et al.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCP5 positive</td>
<td>6</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>HCP5 Negative</td>
<td>0</td>
<td>594</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity : 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity : 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rodriguez-Novoa et al.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCP5 positive</td>
<td>14</td>
<td>1</td>
<td>93%</td>
</tr>
<tr>
<td>HCP5 Negative</td>
<td>0</td>
<td>230</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity : 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity : 99%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HCP5 = HLA complex protein P5 gene
SNP Testing – HCP5 rs2395029

1. Assay components and DNA template

2. Denatured template and annealing assay components

3. Polymerization and signal generation

Legend
- VIC® dye
- FAM™ dye
- Quencher
- Minor Groove Binder
- AmpliTaq Gold® DNA Polymerase

[Diagram showing assay components and DNA template with different color-coded elements]
Should Clinical Laboratories Implement Testing?

<table>
<thead>
<tr>
<th>Drug</th>
<th>HLA allele</th>
<th>HLA carriage rate</th>
<th>Prevalence of diagnosis</th>
<th>Negative predictive value</th>
<th>Approximate number needed to test to prevent ‘1’</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abacavir</td>
<td>B*5701</td>
<td>6–8% Caucasian</td>
<td>8% (includes 3% true hypersensitivity and 2–7% false-positive diagnosis)</td>
<td>100% for patch test confirmed</td>
<td>13</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;1% African/Asian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5% African–American</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol</td>
<td>B*5801</td>
<td>9–11% Han Chinese</td>
<td>1 out of 250 to 1 out of 1000</td>
<td>100% in Han Chinese</td>
<td>250</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–6% Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>B*1502</td>
<td>10–15% Han Chinese</td>
<td>&lt;1–6 out of 1000</td>
<td>100% in Han Chinese</td>
<td>1000</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.1% Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>B*5701</td>
<td>As for abacavir</td>
<td>8.5 out of 100,000</td>
<td>99.99%</td>
<td>13819</td>
<td>[25]</td>
</tr>
</tbody>
</table>

The number needed to test to prevent one case of drug hypersensitivity or a related type B adverse drug reaction can have a significant impact on the feasibility of the introduction of a pharmacogenetic test into routine clinical practice. It is dependent on the prevalence of the adverse drug reaction as well as the positive predictive value of the test. Explanatory tables are shown in Table 4.
Self-Assessment Questions

1. A drug is a good candidate for TDM if:
   a) it has a wide therapeutic index
   b) there is a good correlation between dose and blood concentration
   c) the drug is administered orally as needed
   d) there is a good correlation between blood concentration and clinical effect

2. Which of the following is NOT a phase I reaction?
   a) Oxidation
   b) methylation
   c) reduction
   d) glucuronidation

3. Which of the following drug and pharmacogenetic biomarker associations are well established?
   a) Abacavir and HLA-B*5701
   b) Tamoxifen and Cyp2D6
   c) Warfarin and Cyp2C9/VCORC1
   d) A and C
   e) All of the above