Phenotyping vs. Genotyping for Pharmacogenomics: Expanded Roles for LC/MS

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Study: 98,000 deaths each year are linked to medical mistakes

by Rick Weiss
The Washington Post

WASHINGTON - About 98,000 Americans die unnecessarily every year from medical mistakes made by physicians, pharmacists and other health-care professionals, according to an independent report that calls for a major overhaul of how the nation addresses medical errors.

More Americans die from medical mistakes than from breast cancer, highway accidents or AIDS, according to the report released yesterday by the Institute of Medicine, an arm of the National Academy of Sciences. That costs the nation almost $9 billion a year, the congressionally chartered research group concluded.
Limited efficacy/response to drugs already on the market

- ACE inhibitors: 10-30%
- Beta Blockers: 15-35%
- Statins: 10-60%
- Alzheimer’s: 30%
- Analgesics: 80%
- Asthma: 60%
- Cardiac Arrhythmias: 60%
- Depression (SSRI): 62%
- Diabetes: 57%
- Hepatitis C: 47%
- Incontinence: 40%
- Migraine (acute): 52%
- Migraine (prophylaxis): 50%
- Oncology: 25%
- Rheumatoid arthritis: 50%
- Schizophrenia: 60%
Emerging area in lab medicine:
Personalized medicine
Find Stakeholders!!

Lab    Hosp. Adm    GP    Specialist

Patients
$100,000,000 support for 5 years by NIH.
UCSF Clinical Pharmacogenomics Working Committee Members

- Chair, Dept. Clinical Pharmacy
- Chair, Dept. Medical Genetics
- Lab director, Dept. Lab Medicine
- Director, Research Technologies, Office of the UCSF Dean
- Research faculty, Graduate School of Pharmacology and Pharmacogenomics

Graduate Program in Pharmaceutical Sciences and Pharmacogenomics

- Section chief, Clinical Pharmacogenomics Laboratory
Translation of PGx into clinical practice

Conversion of genotype data directly into clinical management decisions:

1. Selecting drugs that avoids side effects (e.g., HLA-B for hypersensitivity reactions).
2. Selecting drugs that have the highest rate of therapeutic efficacy.
3. Optimizing dosing for maximum efficacy and toxicity avoidance.
Pharmacokinetic effect of CYP 450

Genes Can Have a Big Effect on Drug Metabolism

- Poor metabolizer
- Intermediate metabolizer
- Extensive metabolizer
- Ultrametabolizer

PGx useful

No PGx

Medications

- imipramine
- paroxetine
- haloperidol
- sertraline

% Normal dosage
Tamoxifen for adjuvant breast cancer therapy
Metabolism of tamoxifen


Tamoxifen (TAM)

CYP2D6 (CYP2B6, CYP2C9, CYP2C19, CYP3A)

4-hydroxyTAM

CYP3A4/5 (CYP2C9 + other CYP isoforms)

N-desmethylTAM

CYP2D6

Endoxifen

10-100x more potent than tamoxifen

Low concentrations

Low SERM potency
Phenotype assignments based on metabolic rates

- In vivo studies involving model drugs as substrates: bufuralol, debrisoquine, dextromethorphan, sparteine
CYD 2D6 allele frequencies
Tamoxifen pharmacogenetic testing

- Tamoxifen most effective on individuals with a wildtype 2D6 genotype (highest concentration of endoxifen).
- Individuals with a *3, *4, *5, *6, and *10 allele have reduced metabolism to endoxifen.
- Alternative drugs are becoming available, e.g., aromatase inhibitors and may be preferred in patients with 2D6 polymorphisms.
Tamoxifen therapy and 2D6 *4

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P = .030

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P = .020

Relapse-free time
Disease-free survival
Tamoxifen therapy and 2D6 *10

Log-rank $P = .0032$
Aromatase inhibitor baggage

- Requires ovarian suppression (chemical menopause with Lupron).
  
  - Osteoporosis
  
  - Hypercholesterolemia
  
  - Vaginal dryness and other symptoms of menopause
Tamoxifen/endoxifen levels

Figure 1. Serum levels of tamoxifen, N-desmethyltamoxifen, and the more reactive metabolites: endoxifen and 4-hydroxytamoxifen
Endoxifen correlation to CYP2D6 Genotypes

Endoxifen (ng/mL)

CYP2D6 Genotype Group

- EM/EM
- IM/EM
- IM/IM
- PM/EM
- PM/IM
- PM/PM

Drug inhibitors?

TDM?

Use AIs?

CYP2D6 Genotype Group

EM/EM IM/EM IM/IM PM/EM PM/IM PM/PM
Effect of 2D6 inhibitors on Tamoxefin

- Sertraline, citalopram, celecoxib
diphenhydramine, chlorpheniramine

Relieves hot flashes, but no 2D6 inhibition

SSRIs, paroxetine and fluoxetine used to relieve hot flashes
Tamoxifen pharmacokinetics
Fuchs WS. Arzneim Forsch 1006;46:418-22.

Single oral 30 mg tamoxifen dose

$C_{\text{max}}$: 60 ng/mL
$T_{\text{max}}$: 3.6 h
$t_{1/2}$: 210 h
Steady state (5-7 $t_{1/2}$): 44-61 days
Genotyping vs. phenotyping: tamoxifen

Considering tam use

CYP 2D6 genotyping

PM or IM?

Consider use of AI

EM?

Start tamoxefin

Low endox

Perform TDM after 2-3 m

Avoid 2D6 inhibitors
CYP 2D6 *1/*2
Ok for tam.
Clopidogrel for cardiovascular disease therapy
Common pathway of platelet activation
Platelet aggregation

Scanning electron micrograph of dormant platelets

Activated, aggregating platelets illustrating fibrin strands
Mechanism of action for clopidogrel

Clopidogrel → active metabolite

ADP

Vasopressin → X

Sheer stress

PAF

EPI

TxA₂, 5-HT, Collagen

Thrombin

X

GP IIb/IIIa

Abciximab, tirofiban

Fibrinogen
Pharmacogenomics of clopidogrel
Simon, NEJM 2009;360:363-75

- Used for the prevention of atherothrombotic events in patients after AMI.
- More potent than salicylates to block platelet function
Pharmacogenomic and pharmacodynamic effects for Plavix
PGx of clopidogrel

Death, AMI, stroke
Competing technologies for clopidogrel resistance testing

- **Functional test:** platelet aggregometry: detects pharmacokinetic and pharmacodynamic interactions (Requires drug usage for assessment)
  
- **Pharmacogenomics:** CYP2C19 assess pharmacokinetic interactions alone  
  Germ-line genotyping (no drug dosing required)

- **Therapeutic drug monitoring**  
  (Requires drug usage for assessment)
Clopidogrel loading doses according to platelet function testing in pts with resistance

- Each additional bolus of 600 mg of clopidogrel decreased the number of low responders by at least 35%.
- Despite 2400 mg of clopidogrel, 11 (14%) patients remained low-responders.

Bonello L. et al, Eur Heart J 2008
Can Plavix resistance be reversed? GRAVITAS randomized trial

PCI w/stent

Plavix resistance testing (Verify Now)

- resistant
- sensitive

randomize

75 mg Plavix

150 mg Plavix

Excluded from trial

75 mg Plavix

1 and 6 mo followup for MACE
Clopidogrel TDM for drug titration

• Increasing dosing may not reverse resistance in pharmacodynamic cases (e.g., abnormal platelet receptor)
• Resistance due to reduced metabolism can be overcome by increasing dosing.
• Therapeutic drug monitoring of active metabolite (e.g., by LC/MS/MS) can be used to titrate therapy to “wildtype levels” where optimum clopidogrel dosing was established (through clinical trials)
• Alternate anti-platelet drugs now available (prasugrel), but are more expensive (clopidogrel will go off patent 2011) and have a higher bleeding rate.
Genotyping vs. phenotyping: clopidogrel

Need antiplatelet drug

CYP 2C19 genotyping

PM or IM?  EM?

TDM after 1 week  Clopidogrel 75 mg

Dose based on TDM

Avoid 2C19 inhibitors, PPI debate
Clopidogrel and PPIs
Ho et al. JAMA 2009;301:937-944

FDA Public Health Advisory: Updated Safety Information about a drug interaction between Clopidogrel Bisulfate and Omeprazole
CYP 2C19 *1/*2
150 mg plavix?
Conclusions

• The debate of genotype vs. phenotype will continue without resolution for next decades
• Combination of testing platforms promises to be the most efficacious for personalized medicine
• Genotyping can be performed without drug dosing with phenotyping to follow.
one dose of tamoxifen

150 HK$
one visit to oncologist

1000 HK$
A laboratory test that can determine if tamoxifen is effective in blocking estrogen receptor and prolonging survival of your mother with breast cancer?

priceless
there are some things money can’t buy. for everything else there’s MasterCard.