Recent Advances in Pharmacogenetics

AACC Molecular Pathology: Principles in Clinical Practice

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May 7, 2012
Disclosure Information

Dr. Wu has no relevant financial relationships to disclose.
After this presentation, you will be able to:

1. Learn basic principles of pharmacogenetics
2. Recognize who is driving pharmacogenetic testing
3. Describe the common pharmacogenetic tests in clinical use
4. Describe the clinical decisions made for pharmacogenetic testing
Warfarin
Pharmacokinetics + Pharmacodynamics

- *2/*3: low dose
- *1: high dose
- -1639 A: low dose
- -1639 G: high dose

*VKORC1 Genotype*  
<table>
<thead>
<tr>
<th>Genotype</th>
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<tbody>
<tr>
<td>AA</td>
<td></td>
</tr>
<tr>
<td>GA</td>
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<tr>
<td>GG</td>
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*CYP2C9 Genotype*  
<table>
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<tr>
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<td>8</td>
</tr>
<tr>
<td><em>2</em>3</td>
<td>11</td>
</tr>
</tbody>
</table>
2C9: is *2 and *3 enough?
VKORC1: one SNP or haplotype analysis?
Warfarin dosing – Using 2C9 & VKORC1

CYP2C9 and VKORC1 on Warfarin Stable Dose

<Modified from Caldwell M., CPSC Advisory Committee Meeting, November 14, 2005>  
http://www.fda.gov/ohrms/dockets/ac/05/slides/8>
GWAS for warfarin dosing
VKORC1 Haplotype or -1639 SNP?


<table>
<thead>
<tr>
<th>Group</th>
<th>Predicted dose (mg)</th>
<th>$R^2$ full model</th>
<th>$R^2$ VKORC1</th>
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<tbody>
<tr>
<td><strong>Asian</strong></td>
<td></td>
<td></td>
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<tr>
<td>-1639</td>
<td>4.596</td>
<td>44.23</td>
<td>25.72</td>
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<tr>
<td>VK haplotype</td>
<td>4.596</td>
<td>44.28</td>
<td>25.79</td>
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<tr>
<td><strong>Blacks</strong></td>
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<tr>
<td>-1639</td>
<td>6.35</td>
<td>27.30</td>
<td>4.83</td>
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<tr>
<td>VK haplotype</td>
<td>6.35</td>
<td>27.43</td>
<td>5.11</td>
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<tr>
<td><strong>Whites</strong></td>
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<tr>
<td>-1639</td>
<td>5.678</td>
<td>53.71</td>
<td>26.66</td>
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<tr>
<td>VK haplotype</td>
<td>5.578</td>
<td>54.29</td>
<td>26.30</td>
</tr>
</tbody>
</table>

No difference between -1639 and VKORC1 haplotypes. Blacks not adequately predicted.
Recent study

Accepted for publication in the *Journal of Thrombosis and Haemostasis*


Received Date: 29-May-2011
Accepted Date: 17-Jul-2011
Article type: Letter - to the Editor

3535TT genotype associated with >10 mg/d warfarin vs 3535CC and 3535CT

**ASSOCIATION OF THE C3435T POLYMORPHISM OF THE MDR1 GENE AND THERAPEUTIC DOSES OF WARFARIN IN THROMBOPHILIC PATIENTS**

*Running title:* Polymorphisms in MDR1 and doses of warfarin

**Authors**
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Daniel Dias Ribeiro ‡ - M.Sc. in Medicine
Karina Braga Gomes Borges § - Ph.D. in Pharmaceutical Sciences
Ana Paula Salles Moura Fernandes § - Ph.D. in Parasitology
Ana Lúcia Bruniaíti Godard * - Post-Doc in Animal Genetics
Ethnic variation for VKORC1: UCSF data

- **Asians**
  - H1/H2
  - A/A

- **Hispanic**
  - H7/H9
  - G/G

- **Whites**
  - H7/H9

- **African American**
  - H7/H9
  - H3/H6
  - ??
Tamoxifen
Metabolism of Tamoxifen


Low SERM potency

10-100x more potent than tamoxifen

Low concentrations
Tamoxifen Therapy and 2D6 *4

Relapse-free time

Disease-free survival
Aromatase Inhibitor Baggage

- Requires ovarian suppression (chemical menopause with Lupron).

  - Osteoporosis
  - Hypercholesterolemia
  - Vaginal dryness and other symptoms of menopause
Endoxifen Levels CYP2D6 Genotypes

Drug inhibitors? TDM?

Use AIs?
Effect of 2D6 inhibitors on Tam

Relieves hot flashes, but no 2D6 inhibition

Sertraline, citalopram, celecoxib, diphenhydramine, chlorpheniramine

SSRIs, paroxetine and fluoxetine used to relieve hot flashes
Correlation of endoxifen to outcomes

Threshold level for endoxifen efficacy. Confirmed by cell culture models.
Genotype-guided tamoxifen dosing
Cardiac Drugs: Statins
KIF6 encodes a Kinesin

- Kinesins: a family of dimeric motor proteins involved in the intracellular transport of organelles, protein complexes and mRNAs
- Trp719Arg replaces a non-polar residue with a basic residue near the coiled-coil structure and might affect cargo binding or regulation of the motor domain
Reduction of Coronary Events by Pravastatin
Shepherd et al. Lancet 2002; 360:1623

PROSPER patients with prior vascular disease

719Arg Carriers

- Placebo
- HR=0.66
- P=0.002

- Pravastatin

Noncarriers

- Placebo
- HR=0.94
- P=0.64

- Pravastatin
LDL-C Lowering by Statin Therapy

PROVE IT
- $KIF6$ Carriers
- Noncarriers

Graphs showing LDL (mg/dL) and CRP (mg/L) levels over time for Pravastatin and Atorvastatin treatments.
PGx Testing for Predicting Statin-Induced Myalgia
Statin-induced myalgia

• Investigated genetic variants predictive of muscle side effects to statins.
• Genotyped 37 patients with myalgia (index=1) and 416 without (index=0)
• Myalgia defined as muscle pain with:
  statin initiation
  coincident with dose increase
  resolved with switching to another statin
  resolved with statin d/c
• Recruitment at Hartford CT; Rogosin Institute, NYC, NY; UCSF, SF CA
31 Candidate gene classifications

- Serotonin receptors
- Pharmacokinetic
- Vascular genes
- Fuel processing and energy transfer
- Diseases (e.g., muscular dystrophy)
- Calcium transport
Results

- **COQ2** ($p$-OH-benzoate polyprenyltransferase, enzyme in Q10 biosynthesis)
- **ATP2G1** (calcium transporting ATPase)
- **DMPK** (protein kinase implicated in myotonic dystrophy)
- Six other genes with $p<0.05$ but were not significant after Bonferroni adjustment of multiple genes.
Can coenzyme Q10 reduce the risk of serious side effects from statin medications?

Answer (from Thomas Behrenbeck, M.D., Ph.D.)

Coenzyme Q10 is a substance made naturally by your body. As a supplement, it's usually sold as a capsule and is marketed under brand names such as Co-Q10, Coenzyme Q10, LiQsorb, and Liquid Co-Q10.

Some researchers think that taking a co-Q10 supplement may reduce the risk of serious muscle damage (rhabdomyolysis). And some small reports suggest that muscle and joint aches from statins might be reduced if co-Q10 is taken with a statin. However, no large studies have confirmed this theory, so current guidelines don't recommend routine use of co-Q10 in people taking statins.
Results
Results

Risk for any allele
Prospective study planned

- More extensive questionnaire on myalgia, pain, and psychological general well being.
- Followup visits with CK and lipids

Figure ? Phase IIb Recruitment and Clinical Assessment
You’ll never forget that day. PLAVIX can help keep you from going through it again.
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
Pharmacokinetic & pharmacodynamic effects for Plavix

**Drug levels-PK effect**

**Platelet inhibition—PD effect**
Competing Technologies for Clopidogrel Resistance Testing

- **Functional test**: platelet aggregometry: detects pharmacokinetic and pharmacodynamic interactions (Requires drug usage for assessment, 3 h window for testing, no transportation)
- **Pharmacogenomics**: CYP2C19 assess pharmacokinetic interactions alone
  Germ-line genotyping (no drug dosing required)
- **TDM** (Requires drug usage)
Can Plavix Resistance be Reversed? GRAVITAS Randomized Trial

PCI w/stent

Plavix resistance testing (Verify Now)

resistant

randomize

75 mg Plavix

150 mg Plavix

1 and 6 mo followup for MACE

sensitive

Excluded from trial

75 mg Plavix
Patients with high on-treatment platelet reactivity receiving high- or standard-dose clopidogrel

Cumulative Incidence of Primary End Point, %

Days

Hazard ratio, 1.01; 95% CI, 0.58-1.76; P = .97

No. at risk
High-dose clopidogrel  1109  1056  1029  1017  1007  998  747  54
Standard-dose clopidogrel 1105  1057  1028  1020  1015  1005  773  53
Pharmacogenomics for PAH
Bucindolol

- Nonselective β-blocker with potent sympatholytic properties.
Bucindolol and $\beta_1$-adrenergic receptor polymorphism
Liggett et al. PNAS 2007;1033:11288-93.

P<0.05
Bucindolol and $\alpha_2$-adrenergic receptor deletion

Bristol et al. Circ 2010;3:21-8

$\alpha_2$-del carriers

$\alpha_2$-wt/wt carriers

$P<0.05$
Arca Biopharma update

• Arca Biopharma sought approval for a pharmacogenomic companion test for bucindolol. Received letter from the FDA requiring a clinical trial to assess safety and efficacy.

• Arca is planning a 3000 patient trial of bucindolol vs. metoprolol in patients with the arg 389 in β1 adrenergic receptor polymorphism (~50% of general population).
Eliminating SJS?

Percent of surface area of skin:
- Stevens Johnson Syndrome: <10%
- SJS-TEN overlap: 10-29%
- Toxic epidermal necrolysis: ≥30%

Medications implicated:
- anti-gout agents
- Antibiotics
- antipsychotics
- antiepileptics
- analgesics
- NSAIDS
Genetic Association between HLA & Hypersensitivity

Human Leukocyte Antigen (HLA) = Human Major Histocompatibility Complex (MHC)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug Class</th>
<th>HLA type</th>
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<tbody>
<tr>
<td>Ximelagatran</td>
<td>Anti-coagulant</td>
<td>HLA-DRB1<em>07, HLA-DQA1</em>02</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>Diuretic (ocular conditions)</td>
<td>HLA-B59</td>
</tr>
<tr>
<td>Oxicam</td>
<td>NSAID</td>
<td>HLA-A2, HLA-B12</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anti-epileptic</td>
<td>HLA-B*1502</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Uricosuric (for treatment of gout)</td>
<td>HLA-B*5801</td>
</tr>
<tr>
<td>Abacavir</td>
<td>Anti-retroviral</td>
<td>HLA-B*5701</td>
</tr>
</tbody>
</table>

HLA-B*1502 and Carbamazepine: odds ratio 2504 (South-eastern Asians)

HLA-B*5801 and Allopurinol: odds ratio 580 (Han Chinese)

HLA-B*5701 and Abacavir: odds ratio 960 (Caucasian)
### Table 4. Performance Characteristics of HLA-B*5701 Screening for Hypersensitivity Reaction to Abacavir in the Control Group.

| Subgroup                                | Positive for HLA-B*5701 | Negative for HLA-B*5701 | Total | Performance Characteristic for Hypersensitivity Reaction  
|-----------------------------------------|-------------------------|--------------------------|-------|----------------------------------------------------------
|                                         | number of patients      |                          |       | percent (95% CI)                                         |
| Clinically diagnosed hypersensitivity reaction |                          |                          |       | Sensitivity: 45.5 (33.1–58.2)                           |
| Hypersensitivity reaction               | 30                      | 36                       | 66    | Specificity: 97.6 (96.2–98.5)                           |
|                                         |                         |                          |       | PPV: 61.2 (46.2–74.8)                                   |
|                                         |                         |                          |       | NPV: 95.5 (93.8–96.8)                                   |
| No hypersensitivity reaction            | 19                      | 762                      | 781   | Specificity: 97.1 (95.5–98.3)                           |
|                                         |                         |                          |       | PPV: 60.4 (45.3–74.2)                                   |
|                                         |                         |                          |       | NPV: 95.2 (93.3–96.7)                                   |
| White subgroup                          |                          |                          |       | Sensitivity: 47.5 (34.6–60.7)                           |
| Hypersensitivity reaction               | 29                      | 32                       | 61    | Specificity: 97.1 (95.5–98.3)                           |
|                                         |                         |                          |       | PPV: 60.4 (45.3–74.2)                                   |
|                                         |                         |                          |       | NPV: 95.2 (93.3–96.7)                                   |
| No hypersensitivity reaction            | 19                      | 638                      | 657   | Specificity: 96.9 (95.5–98.0)                           |
|                                         |                         |                          |       | PPV: 47.9 (33.3–62.8)                                   |
|                                         |                         |                          |       | NPV: 100 (99.5–100)                                    |
| Immunologically confirmed hypersensitivity reaction |                          |                          |       | Sensitivity: 100 (85.2–100)                             |
| Hypersensitivity reaction               | 23                      | 0                        | 23    | Specificity: 96.9 (95.5–98.0)                           |
|                                         |                         |                          |       | PPV: 47.9 (33.3–62.8)                                   |
|                                         |                         |                          |       | NPV: 100 (99.5–100)                                    |
| No hypersensitivity reaction            | 25                      | 794                      | 819   | Specificity: 96.4 (94.7–97.6)                           |
|                                         |                         |                          |       | PPV: 46.8 (32.1–61.9)                                   |
|                                         |                         |                          |       | NPV: 100 (99.4–100)                                    |
Hepatitis C
Patients with chronic hepatitis C are treated with pegylated interferon α and ribavirin.

About 50% of patients with HCV genotype 1 achieve sustained virologic response (SVR).

A CC polymorphism upstream of interleukin 28-B is associated with a 2-fold difference in SVR.

New antiviral therapies are expected to be approved later this year.
Caucasians. Similar results seen for Asians and Blacks
Potential therapeutic approaches

Good response C/C IL28B host genotype
- Two-drug regimen (PegIFN + RBV)
  - 24 weeks

Poor response non-C/C IL28B host genotype
- Triple therapy PegIFN + RBV + DAAVs (+/- other DAAVs)
  - 12–24 weeks
- Multi-DAAV only regimen (IFN sparing)
  - 24 weeks
- Triple therapy PegIFN + RBV + DAAV
  - 24–48 weeks
- PegIFN + RBV Multi-DAAV
  - 24–48 weeks
- Multi-DAAV only regimen (IFN sparing)
  - 24–48 weeks

DAAV: directly acting antivirals.
More intensive therapy in this group
Summary

- FDA approval of therapeutics is designed for maximum efficacy and toxicity avoidance for the wildtype patient.
- Pharmacocgenomic testing enables detection of atypical patients necessitating testing of many to benefit the few.
- Implementation of pharmacogenomics requires a teamwork effort of stakeholders (pharmacologists, oncologists, cardiologists, lab medicine, etc).
- Barriers exist with regards to reimbursement, availability of technology, and education.
Self-Assessment Questions

1. Which medications are prodrugs requiring activation by cytochrome P450 system?
2. Which are the polymorphisms in CYP 2C9 and VKORC1 that is associated with warfarin sensitivity?
3. Which 2C19 genotype is associated with hyperactivity for some substrates?