Warfarin Pharmacogenetics in the Asian Population

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Objectives
- Identify common genetic factors that influence warfarin dose requirements
- Compare frequencies of CYP2C9 and VKORC1 variant alleles among races
- Describe the roles of genetic factors in determining warfarin responses in the Asian population
- Be able to use a warfarin pharmacogenetic dosing algorithm

Warfarin: A Problem Drug
- A widely used oral anticoagulant
- Among the top 10 drugs with the largest number of serious adverse events reports in FDA’s Adverse Event Reporting System
- Asians treated with warfarin for afib are 4 times more likely to develop intracranial hemorrhage compared with Caucasians treated with warfarin for afib

Variable Dose Requirements

Mean dose: 5.2 mg/d
n = 186 Caucasians
-16x dose variability

Reported variability in Asians: 0.8 mg/day to 11 mg/day

Unpredictable individual warfarin dose requirement!
Factors Affecting Warfarin Dose Variability

- Non-genetic factors
  - Age
  - Smoking/EtOH
  - Diseases
  - Race/ethnicity: Asians generally require a lower warfarin dose than Caucasians and African-Americans

- Genetic factors: CYP2C9, VKORC1

CYP2C9

- A major hepatic enzyme that metabolizes S-warfarin
- Two common non-synonymous genetic polymorphisms are clinically important

<table>
<thead>
<tr>
<th>Allele</th>
<th>NT change</th>
<th>AA change</th>
<th>Enzyme activity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>*2</td>
<td>430G&gt;T</td>
<td>Arg144Cys</td>
<td>60-70%</td>
</tr>
<tr>
<td>*3</td>
<td>1075A&gt;C</td>
<td>Ile359Leu</td>
<td>5%</td>
</tr>
</tbody>
</table>

<sup>a</sup>: percent of normal enzyme activity
NT; nucleotide, AA; amino acid

Daily Warfarin Dose Requirement by CYP2C9 Genotype

JAMA. 2002;287:1690-1698
**CYP2C9 Variant Allele Frequency (%)**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Caucasians</th>
<th>AA</th>
<th>Chinese</th>
<th>Malays</th>
<th>Indians</th>
</tr>
</thead>
<tbody>
<tr>
<td>*2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>*3</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

AA: African-Americans


**VKORC1**

- Vitamin K epoxide reductase
- Warfarin target protein

- Non-synonymous SNPs (Asp36Tyr, Val45Ala, Arg58Gly, Leu128Arg) have low minor allele frequency

**VKORC1 Gene**

- Five non-coding SNPs in linkage disequilibrium (LD) form two common haplotypes that are associated with warfarin dose requirement

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>-4931</th>
<th>-1639</th>
<th>1173</th>
<th>1542</th>
<th>2255</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>C</td>
<td>A</td>
<td>T</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>B</td>
<td>T</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>C</td>
</tr>
</tbody>
</table>

**Haplotype and Warfarin Dose Requirement**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>A/A</th>
<th>A/B</th>
<th>B/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin Dose (mg/d)</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

**NEJM 2005;352:2285**
**VKORC1 in Hong Kong Chinese**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Total (n=69)</th>
<th>AA (n=53)</th>
<th>AB (n=14)</th>
<th>BB (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>96</td>
<td>62</td>
<td>62</td>
<td>94</td>
</tr>
</tbody>
</table>

**VKORC1 mRNA Expression by Haplotype**

![Graph showing mRNA expression by haplotype]

**VKORC1 Haplotype Frequency (%)**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Caucasians</th>
<th>AA</th>
<th>Chinese</th>
<th>Malays</th>
<th>Indians</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>37</td>
<td>14</td>
<td>87</td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td>B</td>
<td>58</td>
<td>49</td>
<td>9</td>
<td>30</td>
<td>82</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>62</td>
<td>96</td>
<td>95</td>
<td>94</td>
</tr>
</tbody>
</table>

**VKORC1 and Race**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
<th>ALL (*1/*1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td></td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>African Americans</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
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<td>NEJM 2005;352:2285</td>
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<tr>
<td>Indians</td>
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<td>NEJM 2005;352:2285</td>
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</tbody>
</table>

**Pharmacogenetics and Genomics 2005;15:687**

**NEJM 2005;352:2285**

**Clin Pharmacol Ther 2006;79:197**

**Clin Pharmacol Ther 2006;80:169**

**Pharmacogenetics and Genomics 2006;16:101**
**FDA Revised Warfarin Label**
- “Identification of risk factors for bleeding and certain genetic variations in CYP2C9 and VKORC1 in a patient may increase the need for more frequent INR monitoring and the use of lower warfarin doses…”
- “The lower initiation doses should be considered for patients with certain genetic variations in CYP2C9 and VKORC1 enzymes…”

**August, 2007**

**Genetic influences on Warfarin Responses**

**Dosing Algorithm**
- Several groups have developed warfarin dosing algorithms by using linear regression modeling
- Treated stable warfarin daily dose as a dependent variable, and non-genetic and genetic variables as explanatory variables
- $R^2$ ranges 50-60%.

**www.WarfarinDosing.org**
- A publicly available warfarin dosing algorithm
- Based on data from over 1000 patients
- May be used for “refinement” of warfarin dose in patients whose genotype information is available after 1-4 doses of warfarin
  - $R^2$ after the 4th warfarin dose is ~80%.
The Couma-Gen study

- Prospective, blinded, randomized trial comparing genotype-guided (CYP2C9 and VKORC1) vs. standard warfarin dosing in 200 patients initiating warfarin

- Primary endpoints:
  - Per-patient percentage of out-of-range INRs in 3 months or to the end of warfarin therapy, whichever came first

Circulation 2007;116:2563
The Couma-Gen
- # of INRs/pt was lower in PGX group than in STD group (7.2 2.3 vs. 8.1 3.5, p=0.06)
- # of Δ doses/pt was lower in PGX group than STD group (3.0 2.0 vs. 3.6 2.0)
- Time to stable INR was not reported
- Design issues: Use of nomogram, masking, endpoints, single center
- NIH-sponsored multi-center trial is underway

Warfarin Pharmacogenetic Tests
- In vitro Diagnostic Tests approved by the FDA
  1) The Verigene® Warfarin Metabolism Nucleic Acid Test (Nanosphere Inc.)
  2) Infiniti 2C9-VKORC1 Multiplex Assay (AutoGenomics Inc.)
  3) ParagonDx™ Rapid Genotyping Assay 2C9 & VKORC1 (ParagonDx, LLC)
  4) eSensor® warfarin sensitivity (Osmetech Molecular Diagnostics)
- Assay performance time < 6 hrs
- Cost: $200-400

Summary
- Genetic polymorphisms in CYP2C9 and VKORC1 are associated with inter-individual variability in warfarin dose requirements in Asians
  - CYP2C9: Pharmacokinetics
  - VKORC1: Pharmacodynamics
- Validated pharmacogenetic warfarin dosing algorithms are available
- Close follow up and dose adjustments based on INR responses is still important!