HILOnet Warfarin Test:
DNA Typing for Warfarin (Coumadin®) Prescription
• Licensed by CT Dept of Public Health (CL-0644)

• CLIA registered (ID # 07D1036625 Clinical Laboratory Improvement Amendments) Centers for Medicare and Medicaid (CMS)

• One of the pioneering DNA typing centers in the USA
Infrastructure for DNA Typing
FDA, Medicare, Insurance, Lab Distribution

Distribution
Reimbursement

DNA Typing
Report to Doctor

Clinical Laboratory Partners LLC
Connecticut’s Premier Testing Laboratory

In operation since October 2005
Cytochrome P450 DNA Typing
HILOmet 2D6, 2C9, 2C19
Package insert

Revision of warfarin label expected to include DNA Typing

Nov 2005 - FDA's Clinical Pharmacology Subcommittee of the Advisory Committee on Pharmaceutical Science voted in favor of DNA Typing for Warfarin

Reimbursement
Genomax is already receiving full Medicare reimbursement for our clinical CYP450 DNA Typing HLOmet tests. Also by private insurance. Value of tests recognized.

http://www.fda.gov/OHRMS/DOCKETS/AC/05/slides/2005-4194S1_Slide-Index.htm
First developed as a rat poison
Developed and patented by WARF Wisconsin Alumni Research Foundation,
Discovered by Drs. Link and Campbell at Madison after farmer came with bucket of uncoagulated blood from dead heifer which ate spoiled clover hay

- Pharmaceutical use 1954 (Eisenhower among first users)
- Developed by Bristol-Myers Squibb Company
- Generic since 1997
- Definitive treatment worldwide for the long-term prevention of thromboembolic events
- 23 million prescriptions in the U.S. in 2004 (7 mil Coumadin® + 16 mil Warfarin)
**Drug class**
Anticoagulant, inhibitor Vitamin K, reduces clotting Factors II, VII, IX, X

**Indication**
Atrial fibrillation, Myocardial infarction, Valve replacement, Deep Vein Thrombosis, Pulmonary embolism, Post-surgical

**Clinical complications**
Petechiae, occult/overt GI Bleeding, hematuria, blood transfusions

**Treatment Goals**

**Prothrombin Time (PT):**
Reference 10-15 secs
Usually kept at 2-3x

**International Normalized Ratio (INR):** 2-3
Warfarin Dosing
Wide range of doses (5 mg/wk to 80 mg/wk)

Current co-variants:
- Age
- Weight
- Ethnicity
- Diet
- Herbals
- Vitamin K intake

Role for DNA Typing
Data are convincing and voluminous that drug therapy with warfarin is most likely to be a high impact example of DNA Typing in medicine.
Warfarin over-dosage
CYP2C9 deficiency increases effective dose

CYP2C9

Warfarin

Vitamin K epoxide reductase (VKOR)

Reduced vitamin K

Factors II, VII, IX, X
Proteins C, S, Z

Oxidized vitamin K

Activation

γ-glutamyl carboxylase

Vitamin K Cycle

http://www.fda.gov/OHRMS/DOCKETS/AC/05/slides/2005-4194S1_Slide-Index.htm
CYP2C9: Cytochrome P450 Gene 2C9
Isoenzyme breaks down S-warfarin

**CYP2C9 DRUGS**

**Anticoagulants**
- Warfarin (Coumadin®)

**Angiotensin II Blockers**
- Losartan (Cozaar®)
- Irbesartan (Avapro®)

**Glitazones**
- Rosiglit. (Avandia®)
- Pioglitazone (Actos®)

**Insulin Sensitizers**
- Sulfonylureas
- Glipizide (Glucotrol®)
- Glimepiride (Amaryl®)

**NSAIDs**
- Ibuprofen (Advil®)
- Naproxen (Naprosyn®)

**Antidepressants**
- Fluoxetine (Prozac®)
- Sertraline (Zoloft®)

**PHASE I DRUG METABOLISM**

**PHASE II DRUG METABOLISM**

Lipophilic Drug → CYP2C9 → CYP2C19 → CYP2D6 → Hydrophilic Drug & Metabolite → Kidney → Excretion

**Oxidative Reactions:**
- Hydroxylation
- Demethylation

**Excretion**

**Vitamin K Reductase**
- Vitamin K Oxidized
- Reduced Vitamin K
- CO₂
- O₂
- γ-glutamyl carboxylase
- Functional F, II, VII, IX, X Proteins C, S, Z
- Hypofunctional F, II, VII, IX, X Protein C, S, Z

**Explains ~20% warfarin dosing**

Chromo. 10q24
Independent of VKORC1 status
Clinical DNA Typing at HH and IOL

Drug Metabolizer Status

Personalized Medicine, 3(2), 131-137, 2006

High carrier prevalence of deficient and null alleles of CYP2 genes in a major USA hospital: implications for personalized drug safety

Gualberto Raño1, Greg Makowski1, Andreas Windemuth1, Mohan Kocherla1, Stephen Weiss3, John W Goethe2, Bruce Bower1, Alan HB Wu4, & Paul D Thompson2

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2Genomas, Inc.,
67 Jefferson St., Hartford,
CT 06105, USA
Tel: +1 860 545 4574;
Email: support@genomas.com

Many drugs are metabolized by highly polymorphic cytochrome P450 (CYP) enzymes. Among these enzymes, members of the CYP2 family coded by the CYP2D6, CYP2C9 and CYP2C19 genes are best amenable to the precise prediction of an individual’s innate capacity to metabolize drugs by DNA typing of inherited null and deficient alleles. We determined the frequency of these alleles and the prevalence of their carriers in a New England, USA, tertiary care center to assess underlying population genetic features for the practice of personalized medicine. We determined that 54, 25 and 27% are carriers of at least one deficient or null allele for the CYP2D6, CYP2C9 and CYP2C19 genes, respectively. Furthermore, 6% of individuals are carriers of two null alleles for CYP2D6 and are predicted to have no biochemical activity for this isoenzyme. These results support the implementation of DNA typing of CYP2 genes to diagnose adverse drug reactions and to prevent a substantial number of patients being prescribed drugs they cannot adequately metabolize.
<table>
<thead>
<tr>
<th>Allele</th>
<th>DNA</th>
<th>Protein</th>
<th>Metabolizer Phenotype</th>
<th>Frequency HH HH Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>wild-type</td>
<td>Reference</td>
<td>Normal</td>
<td>86%</td>
</tr>
<tr>
<td>*2</td>
<td>430C&gt;T</td>
<td>Arg144Cys</td>
<td>Deficient</td>
<td>9%</td>
</tr>
<tr>
<td>*3</td>
<td>1075A&gt;C</td>
<td>Ile359Leu</td>
<td>~Null</td>
<td>4%</td>
</tr>
<tr>
<td>*4</td>
<td>1076T&gt;C</td>
<td>Ile359Tyr</td>
<td>Deficient</td>
<td>1% (Far East)</td>
</tr>
<tr>
<td>*5</td>
<td>1080C&gt;G</td>
<td>Asp360Glu</td>
<td>Deficient</td>
<td>African Amer.</td>
</tr>
<tr>
<td>*6</td>
<td>818delA</td>
<td>Frame shift</td>
<td>Null</td>
<td>African Amer.</td>
</tr>
</tbody>
</table>
CYP2C9 DNA Typing Survey
Allele Carrier Frequencies, 121 patients at HH

Time for Steady-State concentrations
Prediction by CYP2C9 DNA Typing

Warfarin (Coumadin®)
Medical imperative for CYP2C9 DNA Typing

Carriers of CYP2C9 deficient alleles

- Higher incidence of supra-therapeutic INR values (>4)
- Required a longer time to achieve stable dosing
- Reported a higher rate of serious or life-threatening bleeding events during initiation of warfarin therapy
- INR measurement-based dose findings are not sufficient
- DNA-guided maintenance dosing is useful

Association Between CYP 2C9 Genetic Variants and Anticoagulation-related Outcomes During Warfarin Therapy
JAMA 2002, Higashi et al 287:1690

CYP 2C9*2 and CYP 2C9*3 are associated with excessive anticoagulation and bleeding
Warfarin Resistance
VKORC1 determines warfarin sensitivity

CYP2C9

Warfarin

Reduced vitamin K

Vitamin K epoxide reductase (VKOR)

Oxidized vitamin K

γ-glutamyl carboxylase

Factors II, VII, IX, X
Proteins C, S, Z

Activation

Vitamin K Cycle

http://www.fda.gov/OHRMS/DOCKETS/AC/05/slides/2005-4194S1_Slide-Index.htm
Codes for Vitamin K Epoxide Reductase (VKOR)

VKOR is receptor for Vitamin K reduction and restoration of biologic activity

Highest expression levels of VKORC1 transcript in liver

Gene identified in 2004


VKORC1
Explains ~20% warfarin dosing
Chromo. 16p11
Independent of CYP2C9 status
# VKORC1 Genotypes and Phenotypes

## 7 alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>DNA Change</th>
<th>Protein Change</th>
<th>Enzymatic Phenotype</th>
<th>Frequency Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>Reference</td>
<td>Reference</td>
<td>Normal</td>
<td>57%</td>
</tr>
<tr>
<td>-1639 G&gt;A</td>
<td>Promoter</td>
<td>Val29Leu</td>
<td>Deficient</td>
<td>43%</td>
</tr>
<tr>
<td>+85 G&gt;T</td>
<td>Val29Leu</td>
<td>Val29Leu</td>
<td>~Null</td>
<td>Rare</td>
</tr>
<tr>
<td>+121 G&gt;T</td>
<td>Ala41Ser</td>
<td>Ala41Ser</td>
<td>~Null</td>
<td>Rare</td>
</tr>
<tr>
<td>+134 T&gt;C</td>
<td>Val45Ala</td>
<td>Val45Ala</td>
<td>~Null</td>
<td>Rare</td>
</tr>
<tr>
<td>+172 A&gt;G</td>
<td>Arg58Gly</td>
<td>Arg58Gly</td>
<td>~Null</td>
<td>Rare</td>
</tr>
<tr>
<td>+1331 G&gt;A</td>
<td>Val66Met</td>
<td>Val66Met</td>
<td>~Null</td>
<td>Rare</td>
</tr>
<tr>
<td>+3487 T&gt;G</td>
<td>Leu128Arg</td>
<td>Leu128Arg</td>
<td>~Null</td>
<td>Rare</td>
</tr>
</tbody>
</table>

~Null mutations result in warfarin resistance (dose>10mg/day)
VKORC1 Promoter Variant –1639 G>A
Allele Carrier Frequencies, N=297

- Non-carriers (GG): 25%
- Single carriers (AG): 19%
- Double carriers (AA): 56%

Sconce et al al  *Blood* 2005; 106: 2329
Carriers of VKORC1 mutations

- VKORC1 coding mutations cause warfarin resistance and multiple coagulation factor deficiency type 2
- Several non-coding SNPs influence warfarin sensitivity at lower mean warfarin maintenance dose (MWD) levels
- -1639G>A promoter homozygous patients (AA) had lower dose requirements than AG or GG

Rost et al, 2004
Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2
Nature 427: 537

Established clinical correlations of VKORC1 and pharmacodynamics of dosage optimization
HILOmet Warfarin Test: Digital DNA
DNA from Blood: PCR, Allele-Specific Assays

DNA Extraction

Multiplex PCR

Wild-type

Mutant

Allele-Specific Primer Extension ASPE
HILOmet Warfarin Test: Digital DNA
Arrays of Micro-Beads, 2 Genes, 12 Alleles
Regression Analysis of Warfarin Dose

Clinical examples

\[ \sqrt{\text{Dose}} = 0.628 - 0.0135(\text{Age}) + 0.0162(\text{height}) - 0.240(\text{Cyp}^*2) - 0.370(\text{Cyp}^*3) - 0.241(\text{VKCOR}) \]


- **Age (years)**
- **Height (cm)**
- 
  - **CYP2C9**
    - *2*: 0, 1, 2
    - *3*: 0, 1, 2
  - **VKORC1**
    - -1639G>A: G/G 1, G/A 2, A/A 3
Regression Analysis of Warfarin Dose

Clinical examples

\[ \sqrt{\text{Dose}} = 0.628 - 0.0135(\text{Age}) + 0.0162(\text{height}) - 0.240(\text{Cyp*2}) - 0.370(\text{Cyp*3}) - 0.241(\text{VKCOR}) \]

Regression Analysis of Warfarin Dose

Clinical examples

Age 30
Ht 170 cm. (5’7”)

CYP2C9 0
VKORC1 0

Recommended warfarin dose
7.3 mg/d
Regression Analysis of Warfarin Dose

Clinical examples

Age 70
Ht 170 cm. (5’7”)

CYP2C9  0
VKORC1  0

Recommended warfarin dose
4.7 mg/d
Regression Analysis of Warfarin Dose

Clinical examples

Age 30
Ht 170 cm. (5’7”)

CYP2C9  *3/*3 (double carrier)
VKORC1 A/A (double carrier)

Recommended warfarin dose
2.2 mg/d
Regression Analysis of Warfarin Dose

Clinical examples

Age 70
Ht 170 cm. (5’7”)

CYP2C9  *3/*3 (double carrier)
VKORC1    A/A (double carrier)

Recommended warfarin dose
0.9 mg/d
### Regression Analysis of Warfarin Dose

**Clinical examples: Summary**

<table>
<thead>
<tr>
<th>Age 30, Ht 170 cm</th>
<th>Warfarin Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9 0, VKORC1 G/G</td>
<td>7.3</td>
</tr>
<tr>
<td>CYP2C9 *3/*3, VKORC1 A/A</td>
<td>2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age 70, Ht 170 cm</th>
<th>Warfarin Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9 0, VKORC1 G/G</td>
<td>4.7</td>
</tr>
<tr>
<td>CYP2C9 *3/*3, VKORC1 A/A</td>
<td>0.9</td>
</tr>
</tbody>
</table>
A 35 year old physician (5’10”, 200 pound) presents with a two week history of spontaneous erythema and left calf tenderness. A diagnosis of superficial thrombophlebitis is made. The patient is taking no medications. An INR is 1.2. Coumadin® (warfarin) is begun, 5 mg daily.

On day 4 an INR is > 10. The patient experiences a severe headache and right upper extremity weakness. A cranial CT reveals a left frontal intracortical hematoma.
Clinical Vignette #1: Questions
DNA-Guided Warfarin Dosing

1. What is the role of CYP2C9 and VKORC1 polymorphisms in this patient?
2. DNA Typing reveals patient is CYP2C9 *3/*3 double carrier and VKORC1 (-1639 G>A) double carrier A/A. What is the role of these polymorphisms?
3. Would earlier consideration of DNA Typing have been clinically indicated?

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Value</th>
<th>Recommended warfarin dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9 *2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CYP2C9 *3</td>
<td>2</td>
<td>2.5 mg/d</td>
</tr>
<tr>
<td>VKORC1 -1639</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Clinical Vignette #2
DNA-Guided Warfarin Dosing

Diagnostic Systems (*HIL Om et Warfarin*) are available for estimating warfarin doses using CYP2C9 and VKORC1 DNA Typing, age, height and weight.

The estimated warfarin dose for a 70 year old patient, CYP2C9 *3 carrier and VKORC1 (-1639 G>A) double carrier (A/A) is how different from a 35 year old patient with wild-type CYP2C9 and VKORC1 genotypes and of the same height and weight?

a. the same  
b. 2 fold lower  
c. 2 fold higher  
d. 4 fold lower (25%)  
e. 4 fold higher
**ADR diagnosis**

- Patient has taken warfarin and manifested bleeding ADRs
- *HILOmet Warfarin* Test used to diagnose likely cause of ADR based on individualized ADR risk
- Allows doctor to clarify etiology of symptoms versus confounding factors (diet, compliance, polypharmacy)
- Rule-out confounders

**ADR prevention**

- *HILOmet Warfarin* allows doctor to prescribe warfarin according to patient’s individualized ADR risk
  - **IF ADR RISK IS LOW:**
    - Prescribe drug w. safeguard
  - **IF ADR RISK IS HIGH:**
    - Reduce drug dose
    - Switch to other drugs
    - Proactively monitor/treat ADRs
In Summary

**DNA-Guided Medicine and HILOmet Warfarin**

**HILOmet Warfarin** is a clinically accurate test for analyzing DNA variants relevant to warfarin dosing and prevention of bleeding.

DNA Typing of 7 VKORC1 + 5 CYP2C9

DNA Typing Predicts 33-50% of variability in warfarin dosing.

DNA Typing + clinical covariates explains 60% of variability in warfarin dosing.

**New paradigm for Drug Therapy**

**DNA Typing for guiding dosage, selecting drugs and preventing adverse drug reactions**

**DNA-guided medicine will be expected by patients**