Pharmacogenetics of Warfarin: Are the Promises Justified

Brian F. Gage, MD, MSc
Associate Professor of Medicine, Washington University
Medical Director, Barnes-Jewish Hospital Anticoagulation Service

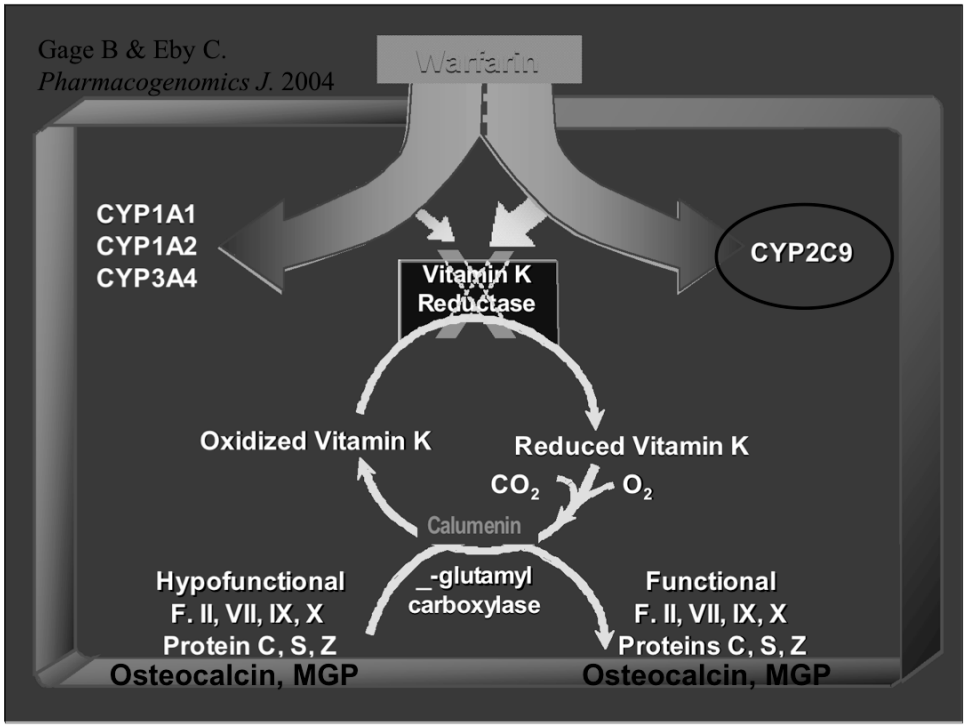
September 27, 2008
8:15-8:40
bgage@im.wustl.edu

Research Funded by R01 HL074724 and AHA

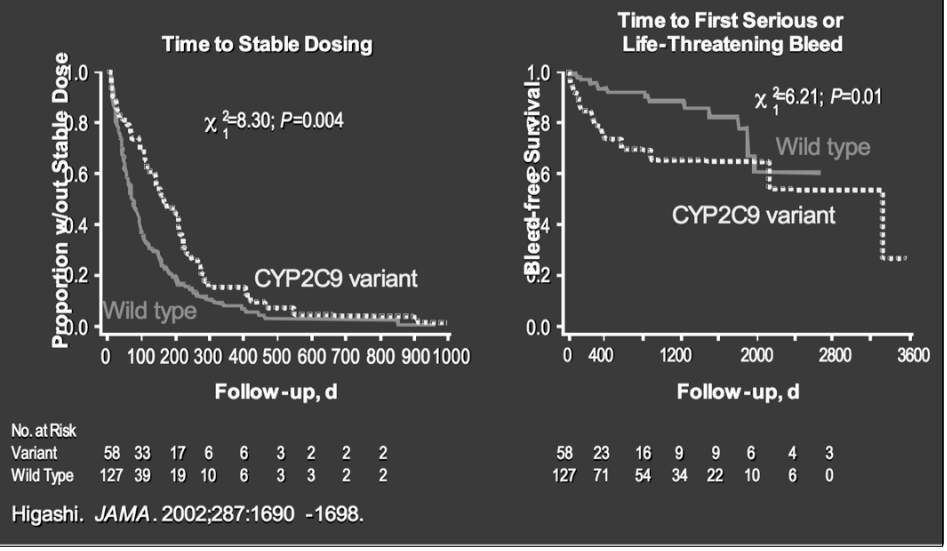
Disclosure: ~5% of research support is provided by industry (Autogenomics or Osmetech)

Outline of Talk

- Polymorphisms that affect warfarin dose
  - *Cytochrome P450 2C9* (*CYP2C9)* SNPs affect warfarin metabolism
  - *Vitamin K epoxide reductase (VKORC1) SNPs* affect warfarin sensitivity
- Pharmacogenetic dosing algorithms
  - Prior to warfarin administration
  - After 3 or 4 days of warfarin administration
  - www.WarfarinDosing.org



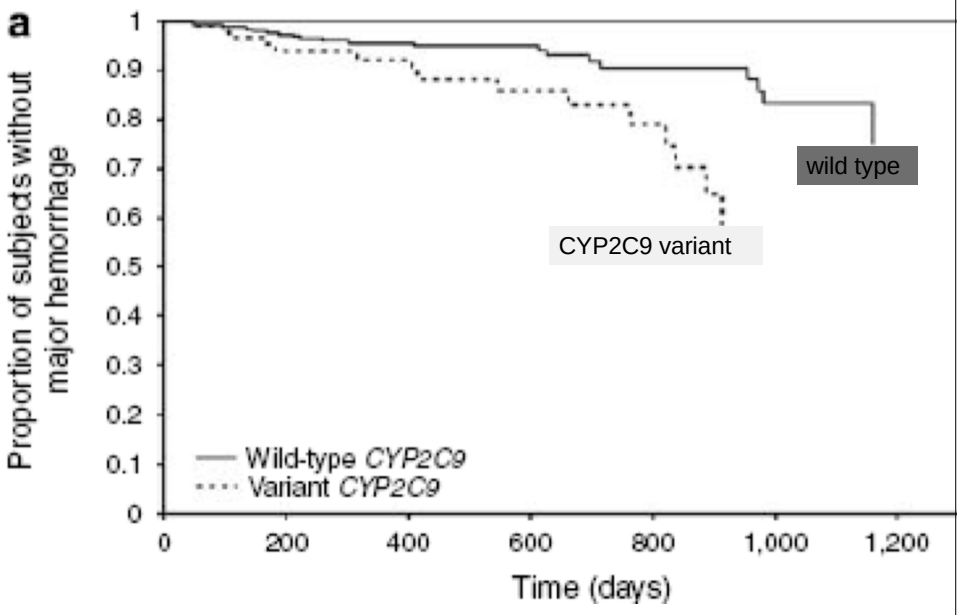
Time to Anticoagulation -Related Outcomes



No. at Risk									
Variant	58	33	17	6	6	3	2	2	2
Wild Type	127	39	19	10	6	3	3	2	2

No. at Risk								
Variant	58	23	16	9	9	6	4	3
Wild Type	127	71	54	34	22	10	6	0

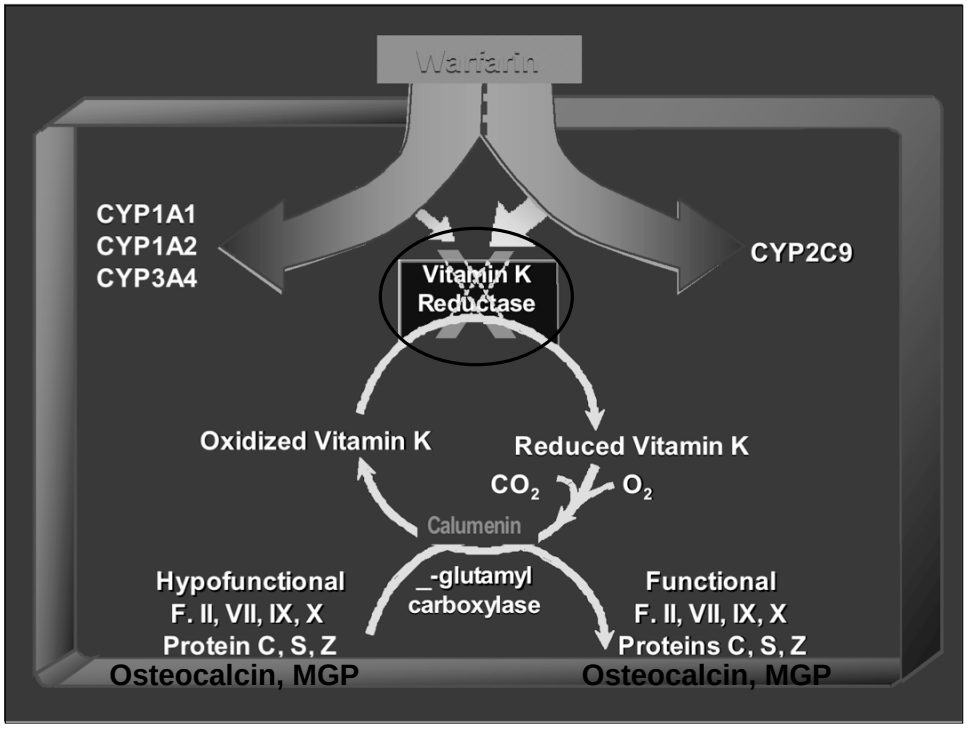
Higashi. *JAMA*. 2002;287:1690 -1698.



Limdi NA. *Clin Pharm Ther* 2007

Summary of CYP2C9 Studies

- CYP2C9*2 & *3 triple the risk of an elevated INR and of adverse events when warfarin is initiated
- CYP2C9*2,*3, and probably*5, *6 variants decrease S-warfarin clearance
- Prospective genotyping facilitates higher initial doses in patients who are CYP2C9*1/*1
- Prospective genotyping allows for lower initial doses in poor metabolizers
  - Which may prevent hemorrhage (1 of 3 small RCTs)
  - Initial dose escalations should be cautious in poor metabolizers



Pharmacogenetics & Warfarin Retrospective Study Goals:

- To determine which *VKORC1* SNPs predict dose
- To understand how to combine these SNPs with CYP2C9 & clinical factors to estimate the therapeutic dose of warfarin

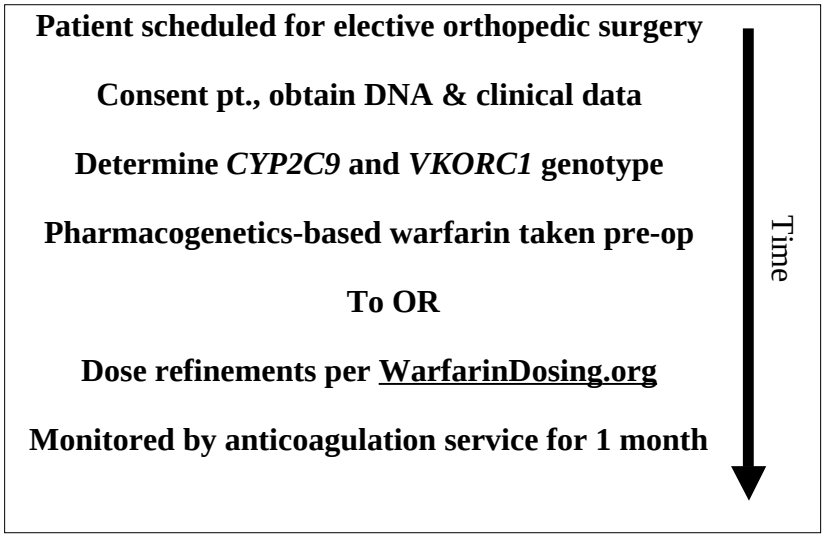
Demographic & Clinical Factors in Retrospective Cohort

Variable		N = 1015
Demographic variables		
	Age, mean (SD), y	65 (14)
	Gender	
	Women, N (%)	362 (36%)
	Men, N (%)	653 (64%)
	Race	
	Caucasian, N (%)	838 (83%)
	African-American, N (%)	153 (15%)
	Other*, N (%)	24 (2%)
	Amiodarone, N (%)	36 (4%)

Results of Stepwise Regression in Derivation Cohort (N = 1015)

Entry	Variable	Effect on Dose	R^2	*P* value
1	VKOR-1639/3673	–28%	25%	<0.0001
2	BSA, per 0.25 m^2	11%	34%	<0.0001
3	CYP2C9*3	–33%	40%	<0.0001
4	Age, per decade	–7%	45%	<0.0001
5	CYP2C9*2	–19%	50%	<0.0001
6	Target INR	11%	51%	<0.0001
7	Amiodarone	–22%	52%	<0.0001
8	Smokes	10%	52%	0.0022
9	AA race	–9%	53%	0.0023
10	Prior DVT or PE	7%	53%	0.0132

Prospective Validation



Prospective Validation (N = 292)

- R^2 = 54%
- Pharmacogenetics-therapy was well accepted
- Frequency of INR > 4 was low
  - However, poor metabolizers can overdose after a dose escalation

Gage BF. *Clin Pharmacol Ther* 2008 Feb

High Accuracy of Genotyping Platforms

Table 5
Accuracy Results (95% Confidence Interval) for *CYP2C9* *2, *3, and *VKORC1* –1639 on 112 DNA Samples*

Platform	*CYP2C9* *2	*CYP2C9* *3	*VKORC1* –1639
INFINITI analyzer Autogenomics	100 (97-100)	100 (97-100)	100 (97-100)
Invader assay Third Wave	100 (97-100)	100 (97-100)	99 (96-100)
Tag-It Mutation Detection assay	100 (97-100)	100 (97-100)	99 (96-100)
Pyrosequencing	99 (96-100)	100 (97-100)	100 (97-100)

Characteristics of the Four Commercial Platforms Evaluated

	INFINITI Analyzer	Invader Assay	Tag-It Mutation Detection Assay	Pyrosequencing
DNA required (ng)	50	250	15	1-5
No. of samples per plate	24	29	24	96
Genotyping time (h)	8	3	8	4
Complexity	Low	Moderate	High	Moderate
Analyzer dimensions (cm)	110 × 60 × 66	38 × 41 × 17	43 × 50.5 × 24.5	111 × 65 × 76

King C et al. *Am J Clin Pathol* 2008

Warfarin Dose Revision Based on INR4

- Goal: To combine genetic and clinical information to estimate the (new) warfarin dose after the initial 4 doses
- Motivation: Improved accuracy of dose refinements should improve INR control
- Genes:
  - *VKORC1* (warfarin sensitivity)
  - *CYP2C9* (S-warfarin metabolism)
- Population: Orthopedic patients

Wash U Derivation Cohorts

	Genetic Cohort (N=86)	Clinical Cohort (N=179)
Age, mean (SD)	60 (14)	59 (15)
BSA, mean (SD)	2.0 (0.3)	2.0 (0.3)
Male, %	49	49
African-Am, %	13	13
Caucasian, %	86	86
Other Race, %	1	1

Wash U + Kaiser Validation Cohorts

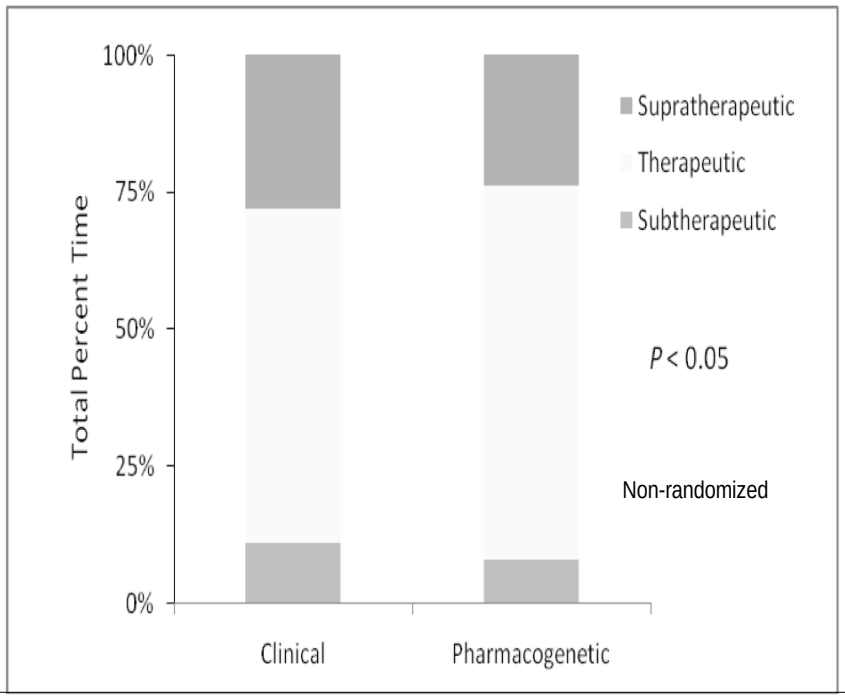
Variables	Genetic Cohort* (N=179)	Clinical Cohort (N=233)
Age in years, mean (SD)	57 (12)	59 (15)
BSA, mean (SD), m^2	2.0 (0.3)	2.0 (0.3)
Male %	48	47
African-American %	9	12
Caucasian %	89	87
Other Race %	1	1

*Includes 38 participants at Kaiser Permanente Colorado

Accuracy of INR4 Algorithms in Validation Cohorts

Algorithm	R^2	< 1 mg error
Clinical	48%	70%
Pharmacogenetic	70%	73%

Lenzini P. et al. *J. Thromb Haemost*. July 2008 (epub ahead of print)



Pharmacogenetic INR_4 Algorithm in the Pooled Orthopedic Cohorts (N = 232)

Entry	Variable	Change in dose, %	R^2 after entry, %
1	Average Doses 1-3	+ 15	54
2	ln(INR_4)	− 20	68
3	*CYP2C9*3*	− 27	71
4	Statin	− 13	73
5	Current Smoker	+ 14	74
6	*VKORC1-1639 A*	− 10	75
7	*CYP2C9*2*	− 10	76
8	ln(EBL)	+ 3	77

Lenzini et al. *J Thromb Haemost* 2008

Findings of Dose Revision Algorithms

- Dose revision (DR) algorithms combine genotype, clinical factors, and initial INR response to predict the therapeutic warfarin dose.
  - INR_3 or INR_4 DR algorithms explain 77% of variability (R^2) in warfarin dose, in derivation cohorts of orthopedic patients
  - R^2 ~63% in medical patients (not shown)
- This approach will be evaluated in a multi-centered, RCT.
  - But the control arm will use a clinical DR algorithm that also is accurate, R^2 ~50%.

Example: www.WarfarinDosing.org

Recommendations on this Web site are based on data from over 1000 patients.
Once information is entered onto the next page, the initial estimate of therapeutic dose explains 53% of the variability in a warfarin dose. If you return to the Web site and enter an INR value after 3 and/or 4 warfarin doses, the dose refinement is even more accurate.

Initial Information

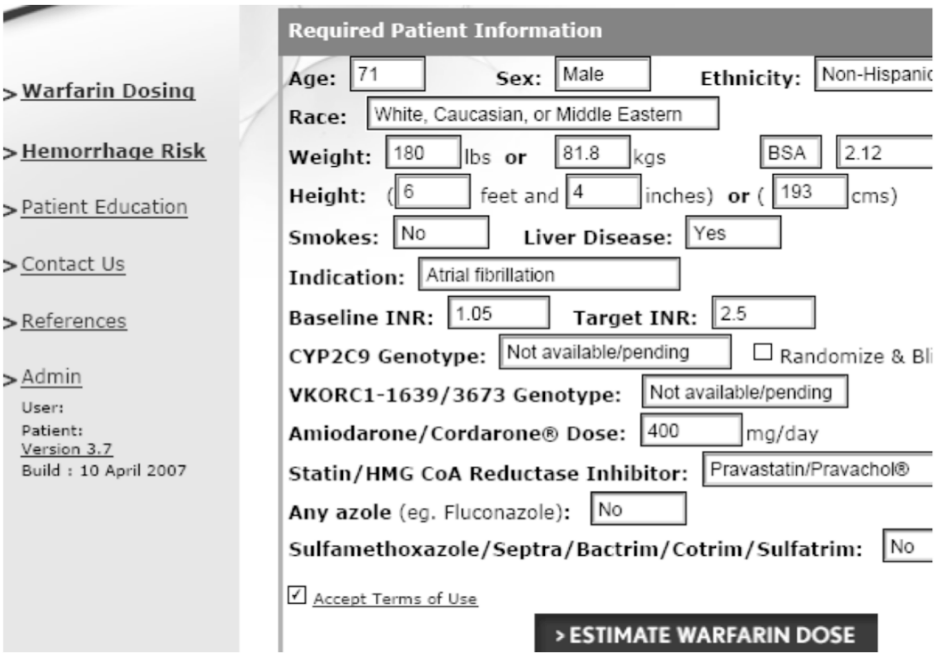
Please provide your information:

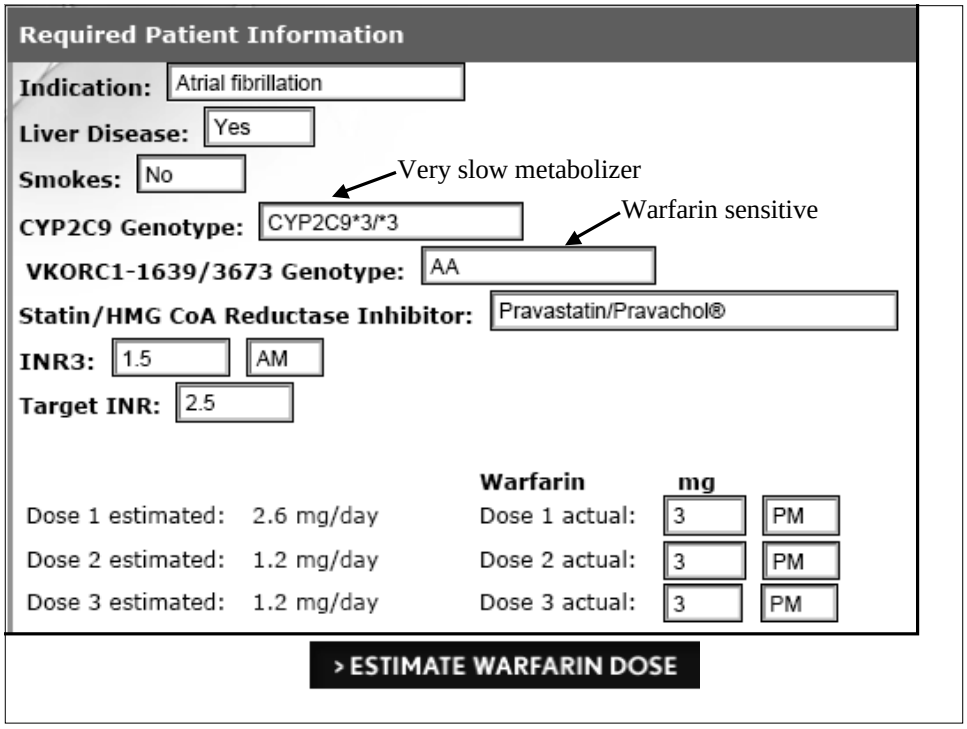
◉ New patient ○ Existing patient

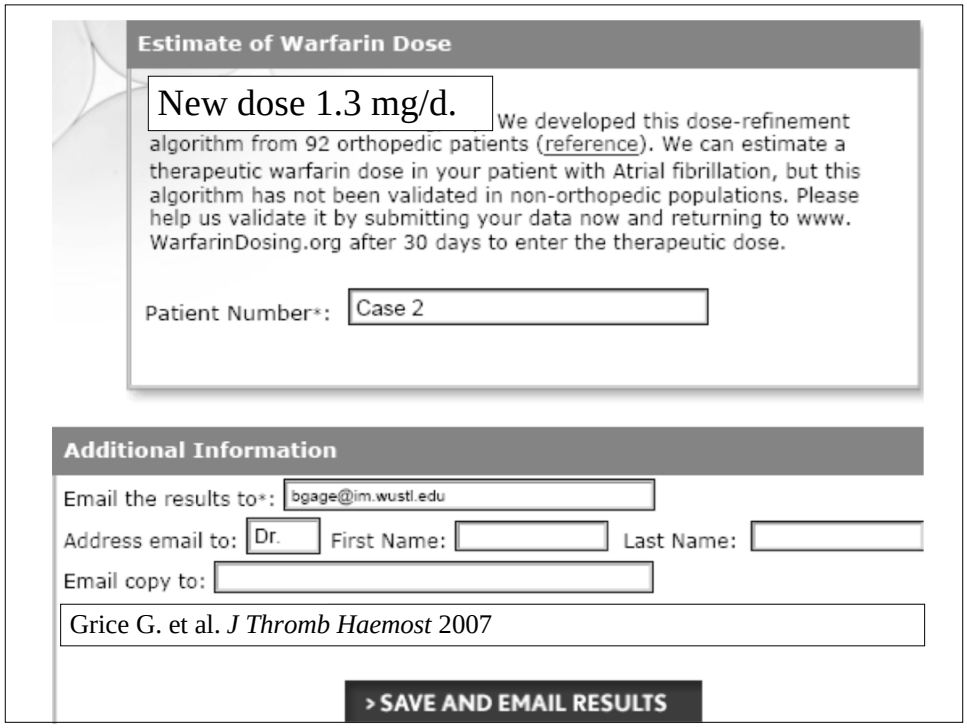
Warfarin doses taken so far*: -Select-

> CONTINUE

*Required




Required Patient Information
Indication: Atrial fibrillation
Liver Disease: Yes
Smokes: No
Very slow metabolizer
CYP2C9 Genotype: CYP2C9*3/*3
Warfarin sensitive
VKORC1-1639/3673 Genotype: AA
Statin/HMG CoA Reductase Inhibitor: Pravastatin/Pravachol®
INR3: 1.5 AM
Target INR: 2.5
Warfarin mg
Dose 1 estimated: 2.6 mg/day
Dose 1 actual: 3 PM
Dose 2 estimated: 1.2 mg/day
Dose 2 actual: 3 PM
Dose 3 estimated: 1.2 mg/day
Dose 3 actual: 3 PM
> ESTIMATE WARFARIN DOSE


Estimate of Warfarin Dose
New dose 1.3 mg/d.
We developed this dose-refinement algorithm from 92 orthopedic patients (reference). We can estimate a therapeutic warfarin dose in your patient with Atrial fibrillation, but this algorithm has not been validated in non-orthopedic populations. Please help us validate it by submitting your data now and returning to www.WarfarinDosing.org after 30 days to enter the therapeutic dose.
Patient Number*: Case 2
Additional Information
Email the results to*: bgage@im.wustl.edu
Address email to: Dr. First Name: Last Name:
Email copy to:
Grice G. et al. *J Thromb Haemost* 2007
> SAVE AND EMAIL RESULTS

Recommendation (for now)

When to recommend *VKORC1* and *CYP2C9* genotyping (select all that apply):

- **outpatient with highly variable INRs**
- **inpatient in the ICU starting warfarin**
- **outpatient starting warfarin & enoxaparin**
- **none of the above**

Summary

- The maintenance warfarin dose can be estimated from clinical and pharmacogenetic factors
  - ~ 50% of the variance in the warfarin dose can be predicted from a regression model using 2 genes and several clinical factors;
    - or from a clinical model that includes $INR_{3 \text{ or } 4}$
  - 63%−70% of the variance can be predicted from a pharmacogenetic model that includes $INR_{3 \text{ or } 4}$
    - *CYP2C9* and *VKORC1* genotypes do not need to be stat for patients to benefit
- www.WarfarinDosing.org is non-profit and publicly available
  - Please try it and let me know what you think

Acknowledgements

- Drs. Eby, Milligan, Grice, Lenzini, Deych, Wadelius, Gatchel, Anderson, Caldwell, Kimmel, Pirmohamed, Limdi, Rane & Johnson.
- Research Funded by R01 HL074724 and AHA