

# Warfarin Pharmacogenomics in the Elderly



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# Objectives

- Consider clinical and genetic factors associated with warfarin dose requirements in the elderly
- Anticipate the role of age, race, body mass, and genetic variables in prescribing treatment with warfarin in the elderly



# Human Genome

- 3,000,000,000 bases (nucleotides) of DNA
  - Adenine (A)
  - Guanine (G)
  - Cytosine (C)
  - Thymine (T)
- Divided into 23 chromosomes
- 25,000 genes
- 99.8% of genome is identical in humans

# Definitions:

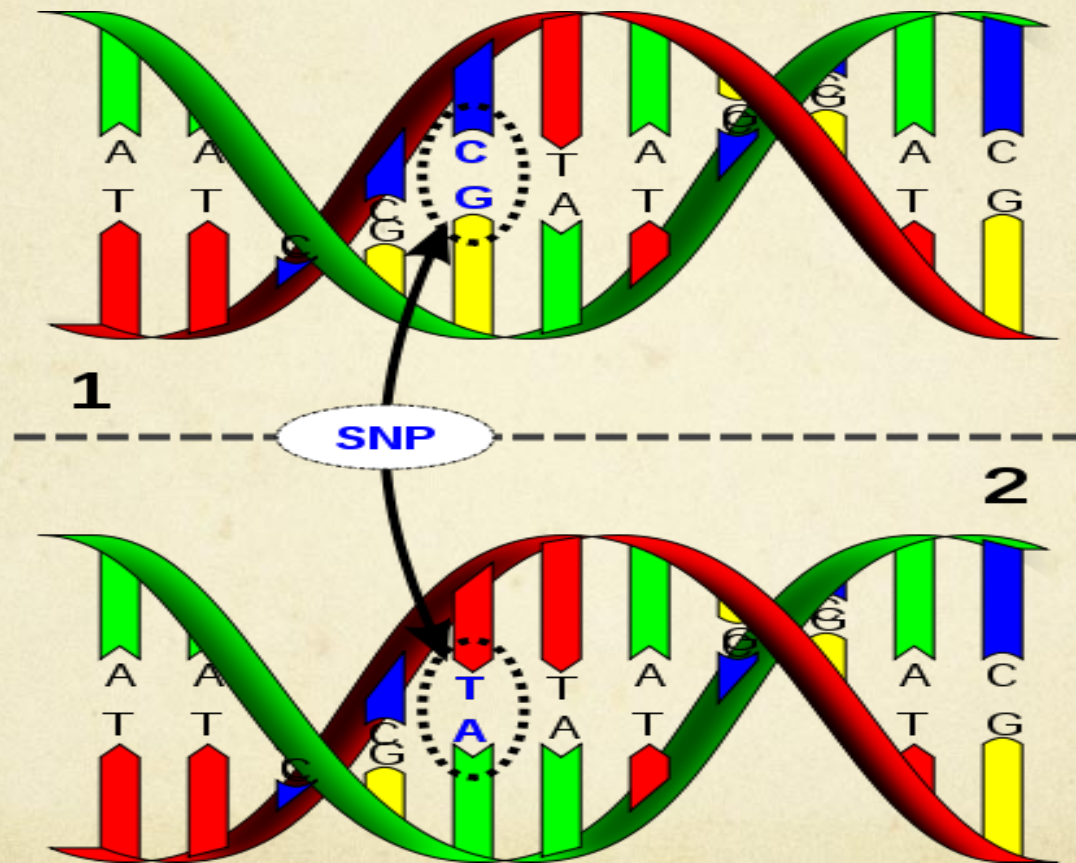
- Gene: DNA sequence on a chromosome that codes for a trait
- Allele: Different versions of the same gene
- Genome: entirety of an organism's genetic information



# Definitions:

- Single Nucleotide Polymorphism (SNP)
  - Variation that occurs with a frequency of at least 1% in the human population due to a single base pair change.
- Tag SNP
  - Representative SNP used to characterize a phenotype that is due to multiple genes that are inherited together
- Haplotype
  - A group of SNPs that are characterized by the Tag SNP and together, contribute to a certain phenotype

# Single Nucleotide Polymorphism (SNP)





# Star-allele Nomenclature

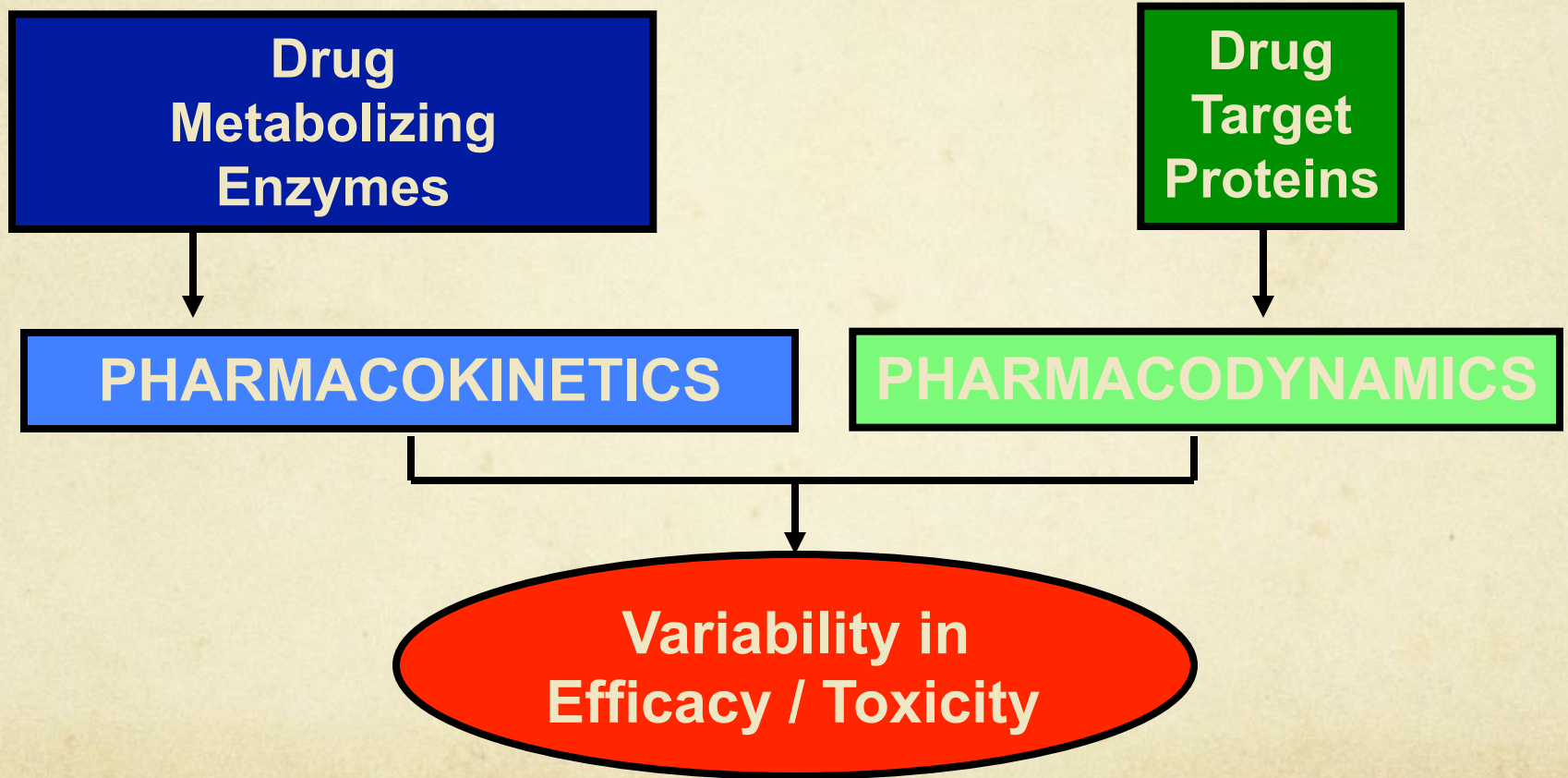
- Designated reference sequence with which polymorphic sites are compared with \*1
  - The first sequence described
  - May not be the most common allele in every ethnic population
- A unique number is assigned (i.e. \*2 or \*3) when a novel variant is identified:
  - That leads to an amino acid substitution
  - Must result in functional change to gene product

# Definitions:

- Pharmacogenetics:
  - Study of how a single gene variation influences drug response.
- Pharmacogenomics:
  - Study of how multiple gene variations influence drug response.
  - Genome wide application of pharmacogenetics



# Pharmacogenomics



# Warfarin Pharmacodynamics

- Inhibits the C1 subunit of the vitamin K epoxide reductase enzyme (VKORC1)
- Results in depletion of reduced vitamin K
- Prevents activation of vitamin K dependent coagulant proteins II, VII, IX, and X
- Also inhibits protein C and S



# Warfarin Pharmacokinetics

- Highly protein bound (99%)
  - mostly to albumin
- Warfarin is given as a racemic mixture
  - Stereo-selective Cytochrome P450 (CYP450) metabolism
  - S-isomer is 3-5 times more potent
  - Metabolized primarily by CYP2C9
    - Hydroxylated to inactive metabolites(92%)

# What We Know-Variability

- Significant inter-patient variability in warfarin dose requirements
- Factors associated with lower warfarin dose:
  - **Advanced age**
  - Polypharmacy (drug interactions)
  - Low body weight
  - Female sex
  - Low dietary vitamin K intake
  - **CYP2C9 variant genotype**
  - **VKORC1 variant genotype**



# What We Know-VKORC1

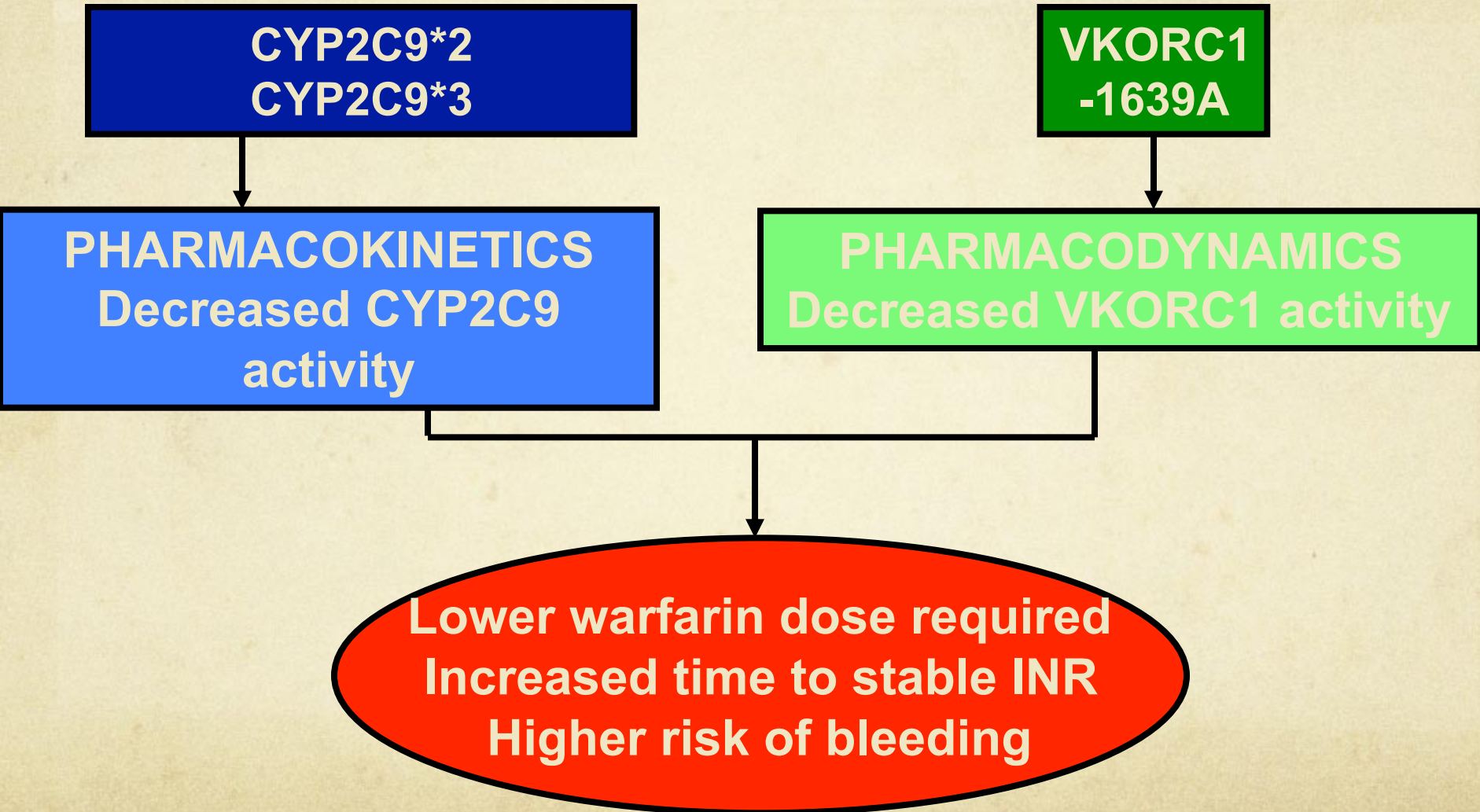
- VKORC1 polymorphisms
  - 10 Common SNPs identified
  - Inherited together in predictable combinations
  - Identified by tag SNP G-1639A
  - Forming two grouped haplotypes
    - A is associated with lower warfarin dose
    - B is associated with higher warfarin dose requirements

# What We Know-CYP2C9

- CYP2C9 polymorphisms
  - CYP2C9\*2, \*3, \*5, and \*6 variant alleles associated with poor metabolism and lower warfarin dose requirements
  - CYP2C9\*2 and \*3 alleles About 35% of Caucasians, but only 3% of African Americans and Asians, carry  $\geq 1$  variant allele.
  - \*5 and \*6 alleles found almost exclusively in African American population
- Polymorphisms in CYP2C9 and VKORC1 account for approximately 50% of warfarin dosing variability



# Warfarin Pharmacogenomics



# FDA approves update to package insert

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**FDA NEWS RELEASE**

**FOR IMMEDIATE RELEASE**  
August 16, 2007

**Media Inquiries:**  
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**FDA Approves Updated Warfarin (Coumadin) Prescribing Information**  
***New Genetic Information May Help Providers Improve Initial Dosing Estimates of the Anticoagulant for Individual Patients***

The U.S. Food and Drug Administration announced today the approval of updated labeling for the widely used blood-thinning drug, Coumadin, to explain that people's genetic makeup may influence how they respond to the drug.

Manufacturers of warfarin, the generic version of Coumadin, are to add similar information to their products' labeling, FDA said.

The labeling change highlights the opportunity for healthcare providers to use genetic tests to improve their initial estimate of what is a reasonable warfarin dose for individual patients. Testing may help optimize the use of warfarin and lower the risk of bleeding complications from the drug.

These labeling updates are based on an analysis of recent studies that found people respond to the drug differently based, in part, on whether they have variations of certain genes.

FDA estimates that 2 million persons start taking warfarin in the United States every year to prevent blood clots, heart attacks and stroke. Warfarin is a difficult drug to use because the optimal dose varies and depends on many risk factors including a patient's diet, age, and the use of other medications.

Patients who take a dose larger than they can tolerate are at risk of life-threatening bleeding. Those who receive too low a dose are at risk of equally dangerous blood clots. Dosing is particularly important at the beginning of therapy, when problems in adjusting the dose can lead to complications such as bleeding.

Updated and expanded in 2010



# Warfarin Dosage from the Official Prescribing Information

- The dose of COUMADIN must be individualized by monitoring the PT/INR. Not all factors causing warfarin dose variability are known. The maintenance dose needed to achieve a target PT/INR is influenced by:
  - Clinical factors including age, race, body weight, sex, concomitant medications, and comorbidities and
  - Genetic factors (CYP2C9 and VKORC1 genotypes).
- Select the starting dose based on the expected maintenance dose, taking into account the above factors. ... If the patient's CYP2C9 and VKORC1 genotypes are not known, the initial dose of COUMADIN is usually 2 to 5 mg per day.
- The patient's CYP2C9 and VKORC1 genotype information, when available, can assist in selection of the starting dose. Table 5 describes the range of stable maintenance doses observed in multiple patients having different combinations of CYP2C9 and VKORC1 gene variants. Consider these ranges in choosing the initial dose.

# Therapeutic Maintenance Dose Based on CYP2C9 and VKORC1 Genotypes

VKORC1	CYP2C9					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
<b>GG</b>	5-7 mg	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg
<b>AG</b>	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg
<b>AA</b>	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg



# What We Know

- Elderly are special subset of population
  - Smaller body size
  - Eat less
  - Polypharmacy
- Elderly meet most of the clinical criteria for lower warfarin dosing
  - Already at risk for bleeding complications without accounting for possible genetic factors
- Elderly are at highest risk to have INR above target range (2-3). Elevated INRs likely to lead to more bleeding events

# Objectives

- Goal
  - Determine if polymorphisms in the genes encoding CYP2C9 and VKORC1 are associated with warfarin dose requirements in patients greater than 70 years of age
- Long-term goal
  - Provide evidence for a new warfarin dosing equation specific for elderly patients in order to improve warfarin therapy in this vulnerable population.



# Methods

- **Inclusion Criteria**

- 100 subjects > 70 years old
- INR goal between 2-3
- $\geq 3$  consecutive clinic visits with INR between 1.8 and 3.2 while on same warfarin dose

- **Exclusion criteria**

- Documented history of liver dysfunction
- ALT or AST levels  $\geq 2$  times the upper normal limit

# Methods-Protocol

- I Obtained written informed consent and performed medical record review
- Buccal cells collected by having patient swish 10mL of Scope© mouthwash for 1 minute and expectorate into 50mL collection tube.
- Interviewed patients about missed warfarin doses, alcohol and tobacco use, OTC medications, and recent changes to medications, and illness within the previous month



# Methods-Protocol

- Additional data obtained from medical records
  - Age
  - Weight
  - Height
  - INR on enrollment
  - Indication for warfarin
  - Past medical history
  - Warfarin dose
  - Concomitant medications

# Methods-Protocol

- **DNA isolated** using puregene kit, Gentra Systems Inc. Minneapolis, MN, USA
- **Genotyping** for CYP2C9\*1, \*2, and \*3, VKORC1 G-1639A
  - Performed using validated, TaqMan real-time polymerase chain reaction assays and direct sequencing using BigDye TerminatorR™ technology (Applied Biosystems, CA, USA)
  - PCR performed on Eppendorf Realplex 4S system using EP Realplex software for analysis



# Methods-Statistics

- Subjects possessing one or two variant alleles will be grouped together and compared to those subjects possessing two wild-type alleles.
- Student's t-test will be used to identify polymorphisms associated with warfarin dose requirements
- A linear regression model will then be used to create a warfarin dosing equation specific for subjects over 70 years of age.

# Results

- Total number of subjects enrolled (42)
- Number of subjects genotyped & entered in database (40)
- Sex
  - 38% (15) male and 62% (25) female
- Ethnicity
  - 95% (38) Caucasian
  - African American 3%
  - Southeast Asian 2%.
- Indication for warfarin therapy
  - 87% (35) atrial fibrillation
  - 7% Venous thromboembolism
  - 6% mitral valve replacement (porcine valves)



# Results

Our warfarin study		Warfarin pharmacogenomics consortium
Reported as Mean $\pm$ SD		Reported as Median and interquartile range
n = 40		n = 5052
Age (years)	80.1 $\pm$ 5.7	41.5% (1,675) age $\geq$ 70
Height (in)	66.2 $\pm$ 4.6	66.14 (62.99-69.29)
Weight (kg)	74.3 $\pm$ 16.95	75.3 (62.0-89.4)
Warfarin dose mg/wk	27.68 $\pm$ 14.27	28 (19.0-38.5)
Warfarin dose mg/day	3.95 $\pm$ 2.03	Not reported
Days at stable dose	73 $\pm$ 70	Not reported
Average INR achieved	2.5 $\pm$ 0.3	Not reported

# Results-Adverse Events

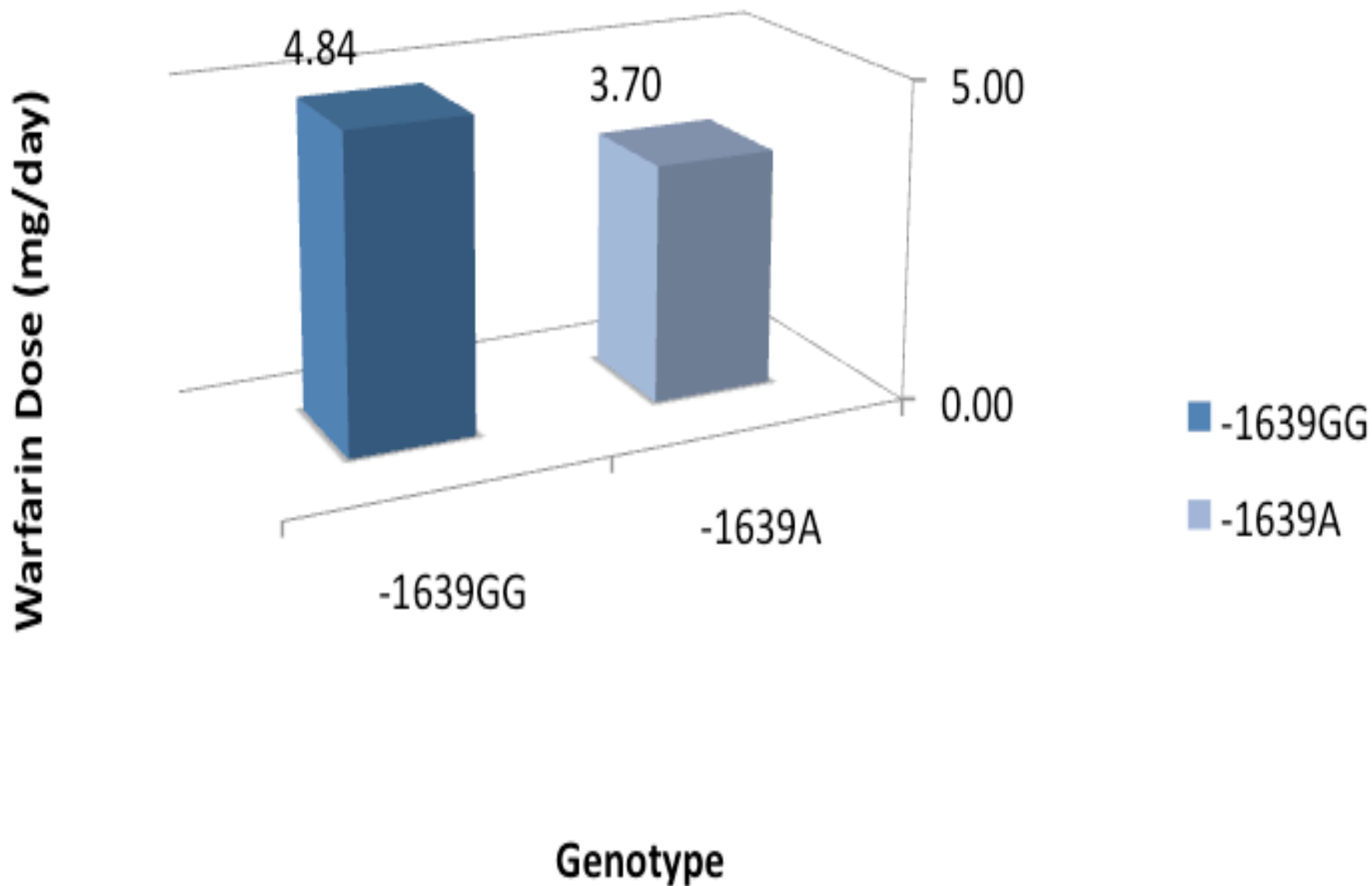
- During period of stable dosing
- 55% (22) patients reported at least one minor/nuisance bleeding event while on warfarin therapy
- 40% (16) reported two or more minor/nuisance bleeding events while on warfarin therapy



# Results

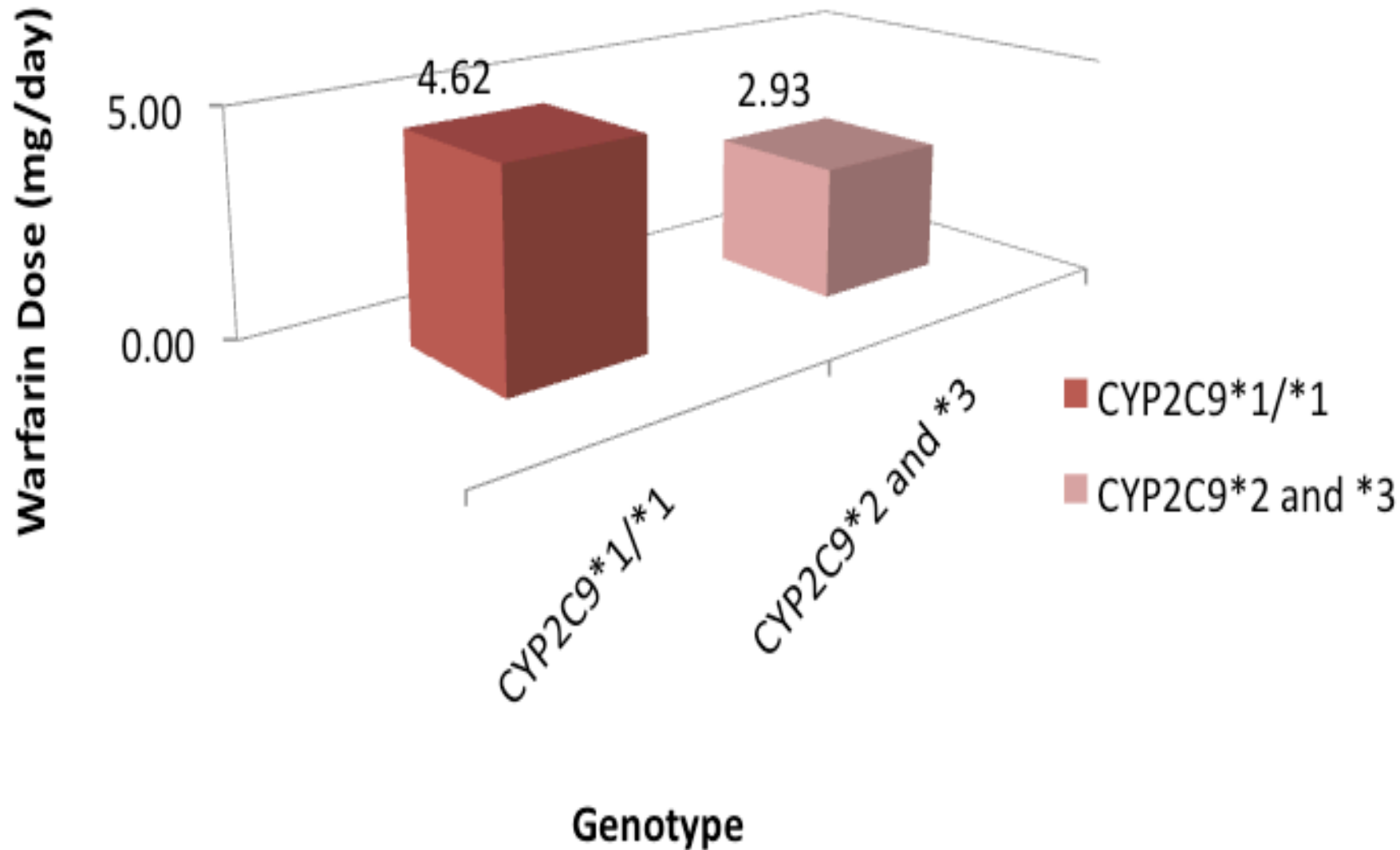
- 23% (9) subjects possess the VKORC1 -1639AA genotype
  - Possession of the VKORC1-1639A allele was associated with lower warfarin dose requirements compared to -1639GG allele carriers.
- 40% (16) subjects possess a CYP2C9\*2 or \*3 variant allele
  - Possession of a CYP2C9\*2 or \*3 allele was associated with lower warfarin dose requirements compared to CYP2C9\*1/\*1.

# Warfarin Dose vs. VKORC1 Genotype





# Warfarin Dose vs. CYP2C9 Genotype



# Results

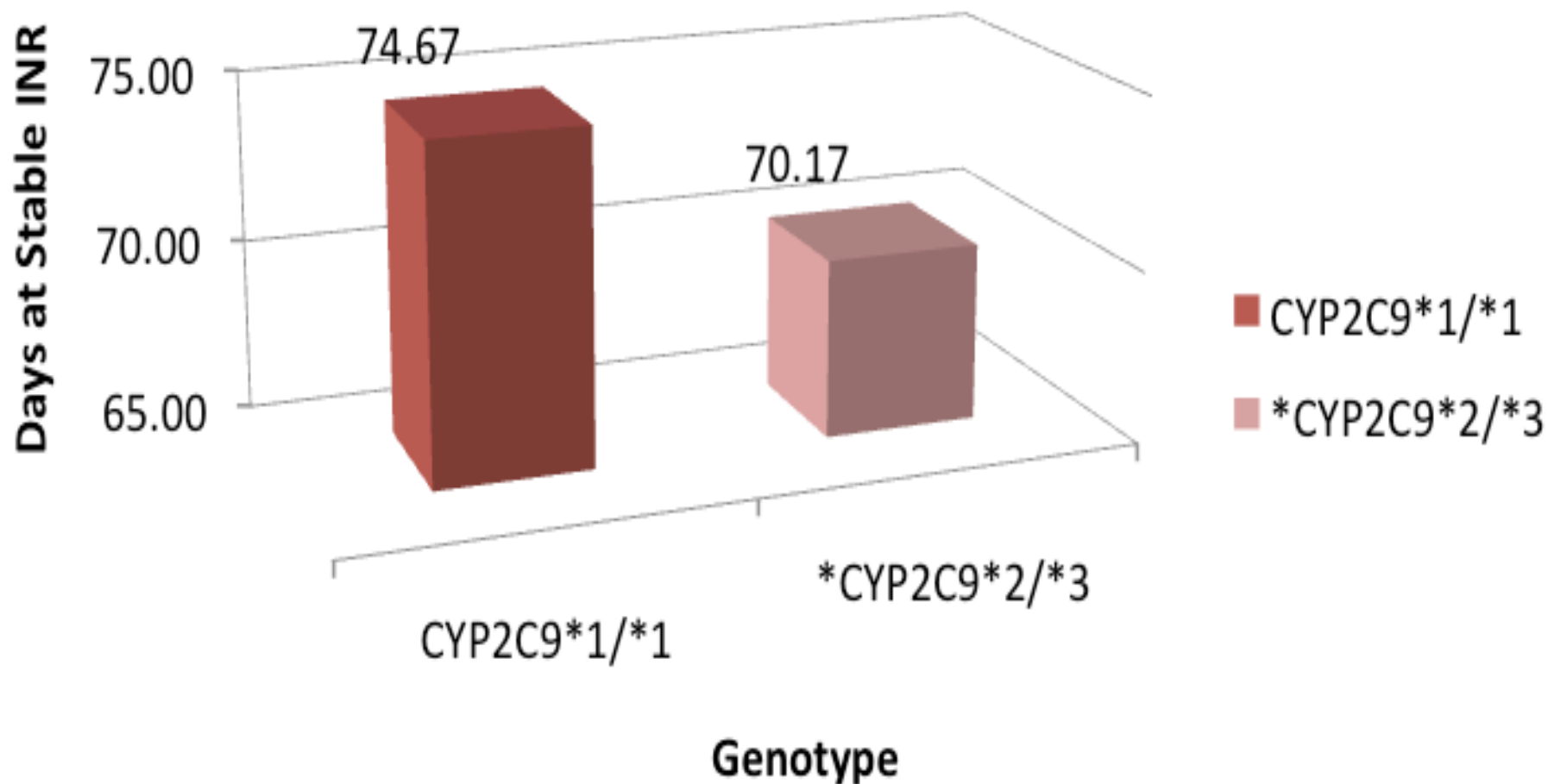
- Empiric dosing for warfarin
  - Initiation dose is  $\leq 5$ mg/day
- All the subjects in this study required  $< 5$ mg/day
  - Possessing wildtype alleles for;
    - VKORC1 – 4.82mg/day
    - CYP2C9 – 4.62mg/day
  - Possessing variant alleles for;
    - VKORC1 – 3.70mg/day
    - CYP2C9 – 2.93mg/day
- **Empiric dose would over-anticoagulate up to 40% of this patient population**



# Results

- Average number of days at stable INR for those possessing a CYP2C9\*2 and/or \*3 allele was 70.17 days
- Average number of days at stable INR for those with the wildtype alleles (\*1/\*1) was 74.67 days

# Days at Stable INR vs. Genotype





# Conclusions

- The current data suggests that possession of the VKORC1 -1639AA genotype and/or a CYP2C9\*2 or \*3 allele is associated with lower warfarin dose requirements in the elderly.

# Limitations

- Ethnicity of study population disproportionately Caucasian (95%)
- Currently limited by small sample size
  - I expect the ethnicities of the study population to become more diverse as recruiting continues and with additional recruiting sites
- Contribution of clinical factors has not been analyzed with, nor compared to the genetic variables' effect on warfarin dose



# Future Directions

- Fills a hole in the current literature
  - My study population only includes subjects  $\geq 70$  years of age
- Completion of this study will provide insight as to the relationship between age, race, body mass, and the evaluated genetic variables, and their relative contribution to warfarin dosing in the elderly.

# Future Directions

- R-warfarin isomer
  - Responsible for about 30%-40% total anticoagulant effect in general population
  - Possible decreased clearance vs. younger patients
  - May account for a larger anticoagulant effect in the elderly
- Can the dosing nomograms be improved upon by including polymorphisms in the genes encoding enzymes involved in R-warfarin metabolism?
  - Role of polymorphisms in CYP450 that metabolize R-warfarin
  - Primarily metabolized by CYP2C19, CYP3A4, CYP3A5, and CYP1A2



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