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Pharmacogenomics of Warfarin

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Abstract
in patients with deep vein thrombosis. Warfarin act like other coumarin-type drugs with similar mechanisms of action acts as an inhibitor of VKORC1; this leads to a reduced amount of Vitamin K available to serve as a cofactor for clotting proteins. Dosing is taken as challenge due to its narrow therapeutics effect. Inappropriate dosing of warfarin can lead to a substantial risk of both major and minor haemorrhage.

Keywords
Warfarin, anti-coagulant, thromboembolic, VKORC1, vitamin-k, dosing.

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Warfarin is most widely used as anticoagulant drugs. It is used to treat patients with Thromboembolic diseases.

GENERAL DEFINATIONS IN PHARMACOGENOMICS: -

• Pharmacogenomics: - It is the study where we get to know how the genes affect the person.

• Pharmacogenetics: - It is the study where we get to know how people respond differently to drug therapy based upon their genetic makeup or genes.

• SNPs: - Single nucleotide polymorphisms in the genetic variation among most of the people. The nucleotide is a single DNA building differentiated by each SNP present in it. Single nucleotide polymorphisms (SNP's) are commonly pronounced as "Snips".

• Various types of SNPs: - They are two types of snips _ non-coding and coding in which coding is again subdivided into non-synonymous SNP and synonymous SNP (silent change. Change of codon, but no change of amino acid in the problem). Non-Synonymous is again divided into Missense SNP (change of codon results in a change of 1 amino acid in the protein) and Nonsense SNP (creates a stop codon in the gene and results in premature truncation of the protein).

• rsID: -Rapid Stain Identification Series (RSID) is designed to detect different human fluids from a variety of samples fast, easy, and reliable which is encountered by forensic laboratories. The United States for the Federal Bureau of Investigation has developed it.

• Allele: - A variant form of a gene is known as an allele. Some genes exist in a variety of different forms, which are located in the same position or genetic locus on a chromosome. Humans have 2 alleles at each genetic locus, where they are inherited each one from a parent.

• Haplotype: - A group of genes that are inherited together from a single parent by the organism.

• Phenotypes: - It is defined by the characteristics of an organism from both genetics and environment through physical and psychological, or a group of organisms having like traits.

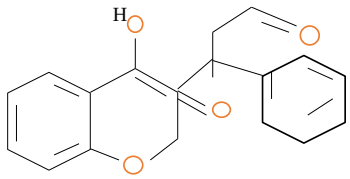
• Loss-of-function/gain-of-function allele: - Loss-of-function mutations, also called inactivating mutations, result in the no function or less function of a gene product. When there is a null allele, it is often called an amorph or amorphic mutation in the Muller's morphs schema.

A type of mutation in which the altered gene product possesses a new pattern of gene expression. Gain-of-function mutations are almost always Dominant or Semi dominant.

• MAF: - Minor allele frequency (MAF) refers to the frequency at which the second most common allele occurs in a given population. SNPs with a minor allele has a frequency of 0.05 (5%)

WARFARIN

STRUCTURE: - Fig -1



MOLECULAR FORMULA: - $C_{19}H_{15}NaO_4$

MOLECULAR WEIGHT: - 330.3 g/mol

Synonyms: -

Warfarin sodium, Athrombin, Athrombin-K, Co-Rax, Coumadin Tab, Coumarins, Panwarfin, Prothromadin, Sofarin, Waran, Warfarin Plus, Warfarin Q.

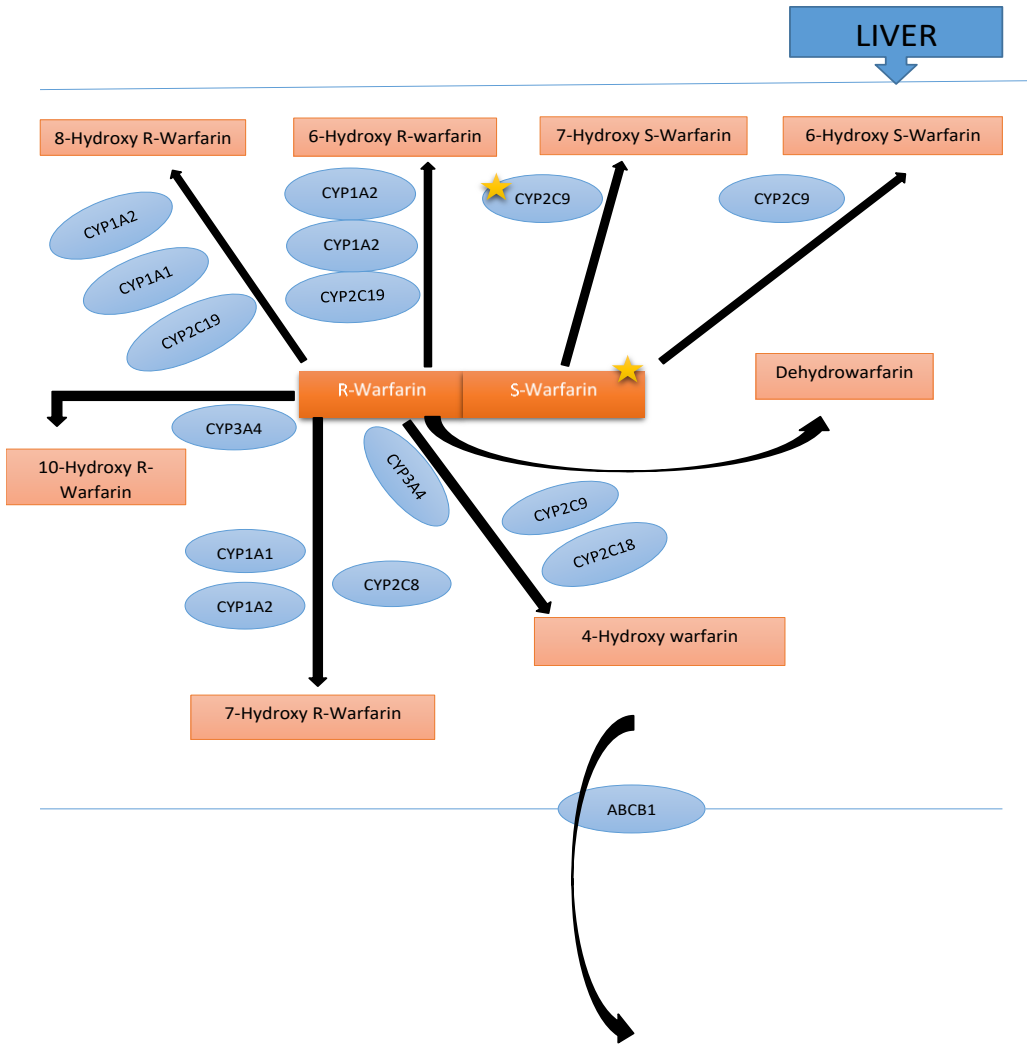
Drug Background: -

Warfarin is one of the most widely prescribed anticoagulant drugs. Chemical name [fig – 1] of warfarin is **2H-1-Benzopyran-2-one, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-**.

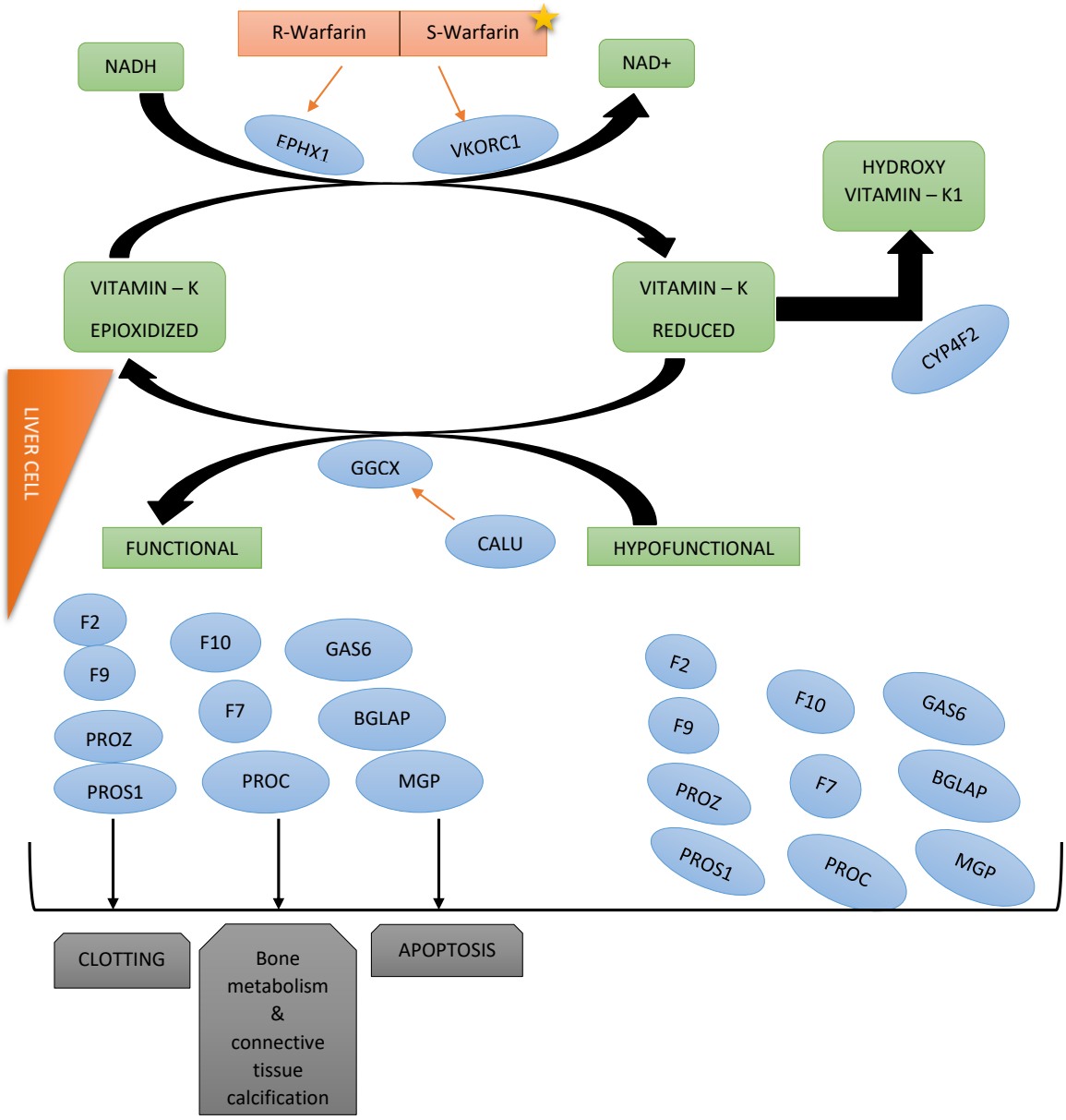
Thromboembolic diseases in patients with deep vein thrombosis, atrial fibrillation, recurrent stroke, or heart valve prosthesis are treated with warfarin. Warfarin act like other coumarin-type drugs with similar mechanisms of action acts as an inhibitor of VKORC1; this leads to a reduced amount of Vitamin K available to serve as a cofactor for clotting proteins. Although effective, warfarin dosing is challenging due to its narrow therapeutic index and a high degree of inter-individual variability in optimal dosing (between 0.6 and 15.5 mg/day). Inappropriate dosing of warfarin can lead to a substantial risk of both major and minor hemorrhage.

PATHWAY: -

Pharmacokinetics = fig – 2 **Schematic diagram of warfarin pharmacokinetics where the enzymes are metabolizing the warfarin.**



Pharmacodynamics (Metabolism) = fig – 3 **Schematic diagram of warfarin metabolism by the enzymes in liver cell**



Description: -

P.K – [figure – 2]

- Warfarin is the most widely used anticoagulant drug. It is highly effective at antagonizing the vitamin K dependent clotting pathway.
- Warfarin is a narrow therapeutic drug and has wide inter-individual variability making dosing problematic.
- Warfarin is a natural product and there are administered as a racemic mixture of the R and S stereoisomers of the drug.
- R-warfarin is 3-5 times less potent inhibitor of the vitamin K epoxide reductase complex, the target of the action, than S-warfarin.
- The different phase 1 enzyme metabolizes the stereoisomers; the predominant metabolism of the S isomer is through CYP2C9 whereas the metabolism of R-warfarin is mainly through CYP3A4 with the involvement of other enzymes such as CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C18 and CYP2C19 as represented in the Warfarin Pharmacokinetics Pathway.
- The metabolism of warfarin in phase 2 has not been well studied and is not pictured in this pathway representation, even though it is known that sulphated and glucuronyl conjugates can be formed.
- However, warfarin has been shown to interact with the ABCB1 transporter in the liver, the elimination predominantly occurs through renal.
- Classical examples of pharmacogenetics can be considered as Warfarin pharmacokinetics and CYP2C9. The 2 most important variants shown to have clinical implications for warfarin dosing and prevention of adverse events are CYP2C9*2 and CYP2C9*3. The need for lower doses of warfarin

is more likely taken by individuals with the 2 and 3 variants because on starting warfarin therapy they take a longer time to reach target INR and have an increased risk of bleeding complications.

- CYP2C9 which has other several polymorphisms have been reported some of which have also been shown to affect warfarin metabolism.
- There are 6 antibiotics they are erythromycin, cotrimoxazole, fluconazole, metronidazole, miconazole, and isoniazid; 5 cardiac drugs they are clofibrate, amiodarone, propafenone, sulfinpyrazone, and propranolol; phenylbutazone; piroxicam; alcohol that only with concomitant liver disease which interacts with warfarin.
- Cimetidine: and omeprazole which potentiates warfarin action, and 3 antibiotics such as griseofulvin, rifampin, and nafcillin; 3 drugs active on the central nervous system (barbiturates, carbamazepine, and chlordiazepoxide); cholestyramine; sucralfate that inhibits warfarin action. The diet which interacts with the clinical outcomes of warfarin is vegetables containing vitamin K, such as spinach and kale, and avocado.
- Recently the VKORC1 gene was identified as the major subunit of the vitamin K epoxide reductase complex has resulted in new candidates for warfarin pharmacogenomics.
- Vitamin K epoxide reductase and the downstream genes are represented in the warfarin pharmacodynamics pathway whose products, are postranslational carboxylated to become Gla-containing proteins by gamma-glutamyl carboxylase (GGCX)
- Gla-containing proteins are involved in hemostasis (coagulation factors F2, F7, F9, F10, Protein C, S, and Z) as well as bone metabolism (BGLAP), tissue matrix (MGP) and apoptosis (GAS6).
- Influence in warfarin sensitivity including 4 mutations that are linked to warfarin resistance is reported because of several variants in VKORC1.
- 1173C in the intron 1 that is more common in high daily dose patients.
- A haplotype of 4 variants in the promoter, introns, and 3'UTR that have been shown to correlate with maintenance dose although no variants have yet been shown to be directly causative.
- A variant of CYP4F2 (rs2108622, V433M) has been shown to be associated with warfarin dose recently.
- The metabolism of vitamin K1 to hydroxyvitamin K1 is catalyzed by CYP4F2 is a primary liver vitamin K1 and removes vitamin K from the vitamin K cycle.
- A counterpart to VKORC1 plays an important role in limiting the excessive accumulation of vitamin K.
- Comparing to CC genotype patients CYP4F2 rs2108622 TT genotype carrying patients require more amount of warfarin approximately 1mg/day.
- Furthermore, CYP4F2 and in addition to functional variants in CYP2C9, VKORC1, and clinical factors, the dosing models showed an improvement in the overall predictability of warfarin dose.
- However, there are also contradictory reports showing that the contribution of this variant to warfarin dose was negligible.

P.D – [figure – 3]

- Warfarin exhibits its anticoagulant effect through inhibition of its target Vitamin K epoxide reductase (VKORC1).
- The two enantiomers of warfarin are both able to inhibit VKORC1, although with different potencies.
- The mechanism of action of warfarin is depicted in the Warfarin Pharmacodynamics Pathway VKORC1 catalyzes the conversion of oxidized Vitamin K to reduced Vitamin K with the help of microsomal epoxide hydrolase.
- Treatment of warfarin blocks this reaction, which leads to a reduction in the pool of reduced Vitamin K that is needed as a cofactor for clotting proteins.
- The downstream genes influenced by the form of Vitamin K are also depicted in the pathway. Gamma-glutamyl carboxylase (GGCX) coverts the product of these target genes is postranslationally carboxylated to Gla-containing proteins.
- Gla-containing proteins are involved in hemostasis (coagulation factors F2, F7, F9, F10, Protein C, S, and Z) as well as bone metabolism (BGLAP), tissue matrix (MGP) and apoptosis (GAS6).
- Its activity is inhibited when the endoplasmic reticulum chaperone protein calumenin (CALU) can bind to the vitamin K cycle.

Variant Annotation: -

Variant	MOLECULES	Association
CYP2C9*1 CYP2C9*3	WARFARIN	CYP2C9 *3/*3 + *1/*3 are associated with decreased dose of warfarin as compared to CYP2C9 *1/*1
CYP2C9*1CYP2C9*59	DICLOFENAC, LOSARTAN, TOLBUTAMIDE	CYP2C9 *59 is associated with decreased enzyme activity of CYP2C9 when assayed with diclofenac, losartan and tolbutamide as compared to CYP2C9 *1.
CYP2C9*1CYP2C9*3	Piroxicam	CYP2C9 *1/*3 is associated with increased Piroxicam's area under the plasma concentration-time curve and decreased oral clearance when exposed to piroxicam in healthy individuals as compared to CYP2C9 *1/*1
CYP2C9*1	Phenytoin	CYP2C9 *1/*1 is associated with decreased plasma concentration when exposed to phenytoin in healthy individuals as compared to CYP2C9 *1/*2.

Table a – variant annotation of the Pharmacogenesis of warfarin

Genotype to Phenotype

Type of finding	Major findings
Clinical	Individuals with CYP2C9*2 and CYP2C9*3 haplotypes have reduced warfarin maintenance dose.
Clinical	Individuals with CYP2C9*2 Metabolism and inactivation of CYP2C9*3 are likely to S-Warfarin
Clinical	VKORC1: -1639G>A variant is associated with reduced maintenance dose of warfarin in caucasians, asians and blacks. It may explain much of pharmacological variability in warfarin therapy.
Clinical	Multiple rare nonsynonymous SNPs in VKORC1 (V29L, D36Y, V45A, R58G, V66M, R98W, L128R) confer warfarin resistance.
Clinical	VKORC138191"(rs61162043)"variant is associated with higher warfarin dose in African Americans.
Clinical	A variant of CYP4F2 (rs2108622, V433M) affects enzyme activity and is associated with warfarin dose,where patient with TT genotype requires
Clinical	Variant rs339097 in CALU predicts higher warfarin dose in African Americans populations, with the G allele of rs339097 associated with 14.5% higher therapeutic warfarin dose.
Clinical	rs11676382 in GGCX was shown to be a significant (p=0.03) predictor of residual dosing error and was associated with a 6.1% reduction in warfarin dose (95% CI: 0.6%-11.4%) per G allele.

Table b – Genotype to Phenotype of Pharmacogenesis of warfarin

Dosing algorithms [fig – 4]

IWPC warfarin pharmacogenetic dosing algorithm

5.6044
- 0.2614 x Age in decades
+ 0.0087 x Height in cm
+ 0.0128 x Weight in kg
- 0.8677 x VKORC1 A/G
- 1.6974 x VKORC1 A/A
- 0.4854 x VKORC1 genotype unknown
- 0.5211 x CYP2C9*1/*2
- 0.9357 x CYP2C9*1/*3
- 1.0616 x CYP2C9*2/*2
- 1.9206 x CYP2C9*2/*3
- 2.3312 x CYP2C9*3/*3
- 0.2188 x CYP2C9 genotype unknown
- 0.1092 x Asian race
- 0.2760 x Black or African American
- 0.1032 x Missing or Mixed race
+ 1.1816 x Enzyme inducer status
- 0.5503 x Amiodarone status
= Square root of weekly warfarin dose**

**The output of this algorithm must be squared to compute weekly dose in mg and divided by 7 to get the daily dose.

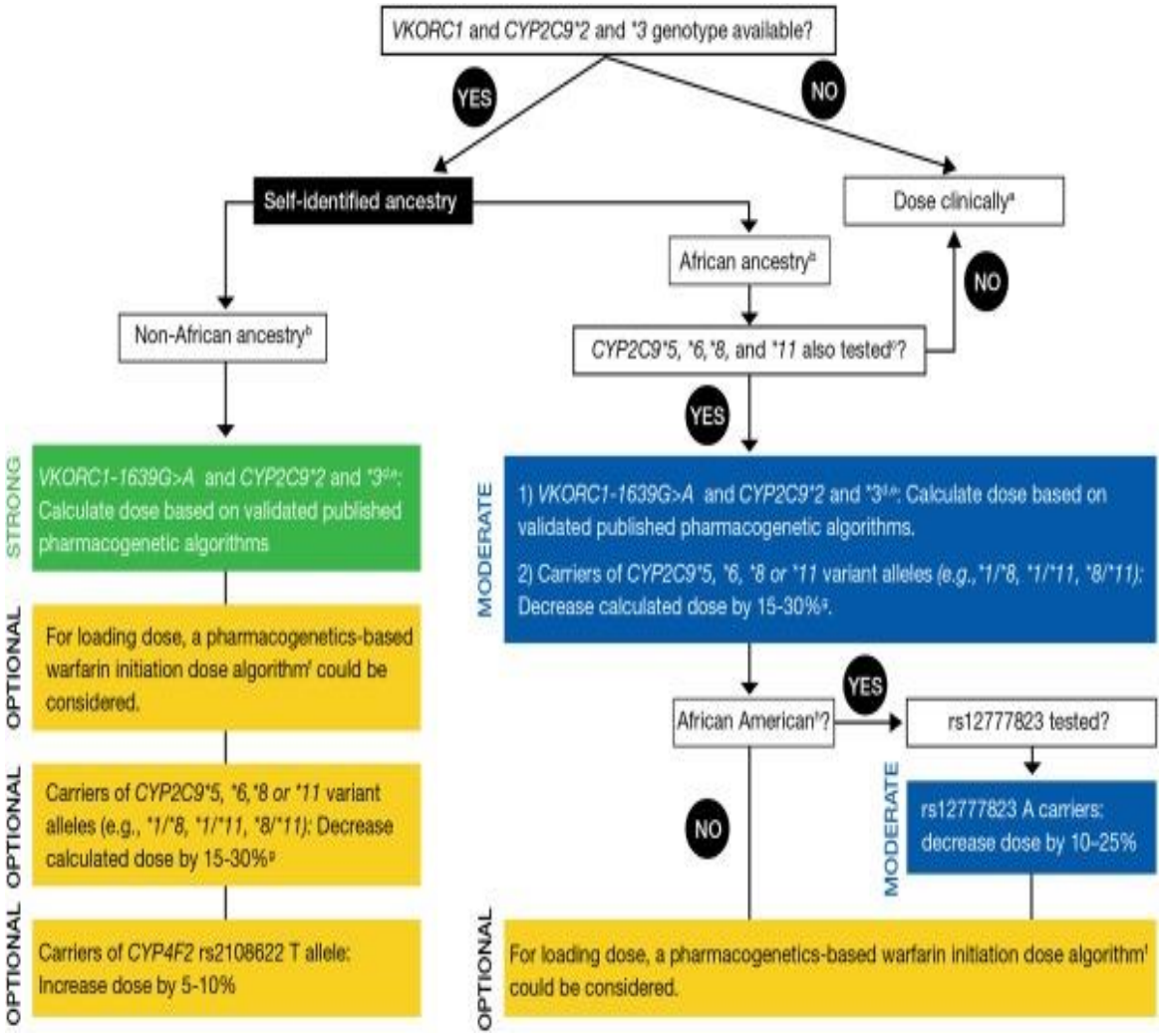


Fig-4 Dosing calculation according to the genes in a particular individual.

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