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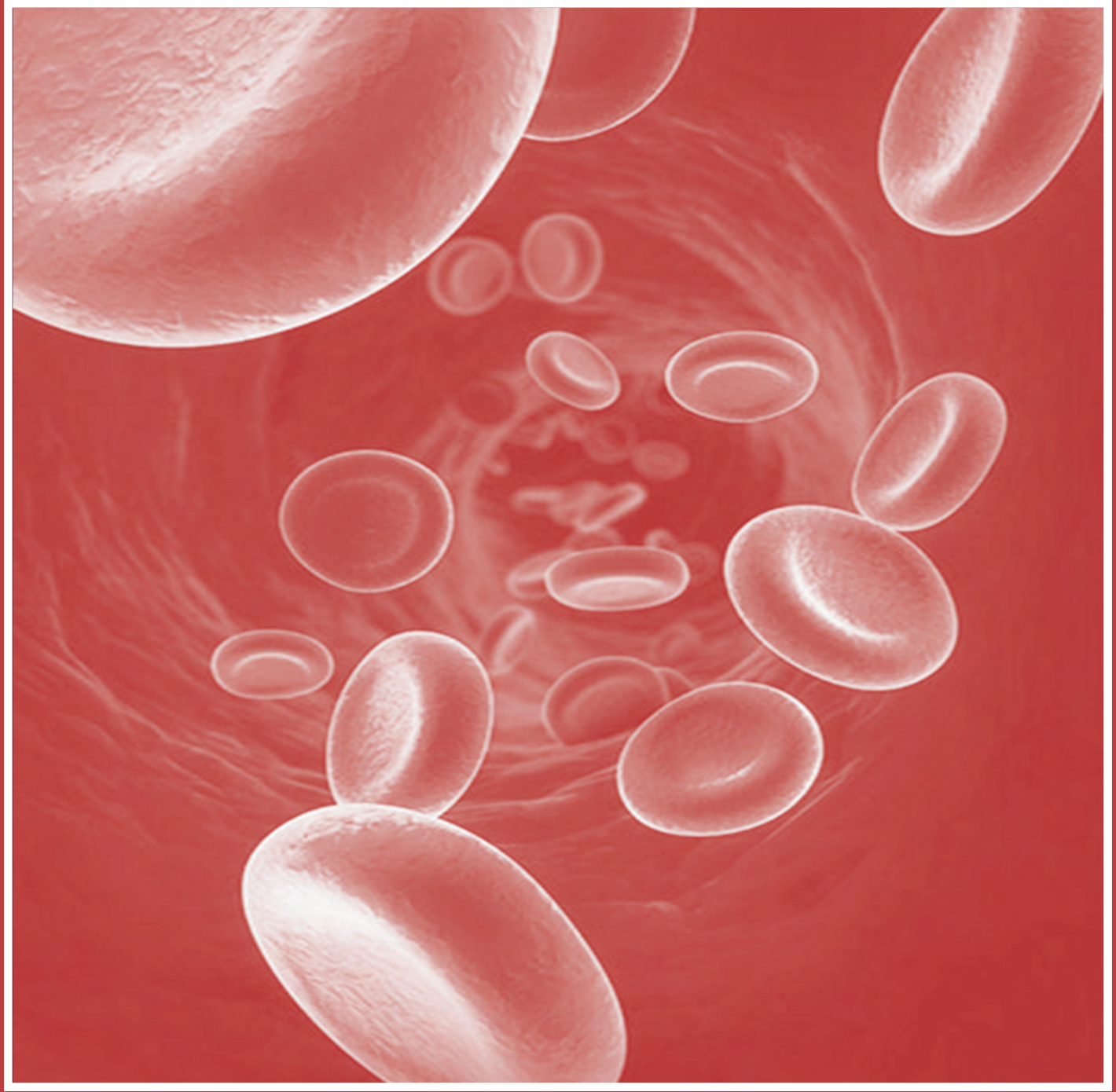

Swachh Bharat Abhiyan
at
Social League School

World Yoga Day

Warfarin pharmacogenetics: Implications for personalized medicine

Tejasvita Gaikwad

Summary

Warfarin (Coumadin), a derivative of coumarin, is a commonly prescribed oral anticoagulant in the world. Although warfarin is indispensable for treatment of various clinical conditions, due to its narrow therapeutic index, high inter- and intra-individual variability in dosage and a high risk of bleeding or thrombosis due to over- or under coagulation, it has remained one of the most challenging medications to manage despite over 60 years of experience with the drug. It is one of the top ten drugs related to adverse drug events and hospitalization.

Of late, considerable effort has been made to understand the association of genetic and non genetic factors on the warfarin dosage or other associated clinical manifestations. Both genetic and non genetic factors were found to have varied contribution to dose variations of warfarin and adverse effects. However, the contribution of genetic factors has been shown to be quite significant. With regard to marked ethnic variations in the allele frequencies of genetic markers, the knowledge of the allele frequency in a population is essential prior to embarking on any pharmacogenomic studies. Till date several reports are available on predicating warfarin dosing based on genetic and non genetic factors from worldwide populations, but it is averagely studied in Indian patients. Studies in this area will improve the management of this challenging therapy by minimizing warfarin induced adverse drug reaction in Indian patients.

Introduction

Pharmacogenomics is a major contributor to the personalized medicine. It has become an integral part of modern drug development and treatment. It intends to enhance the likelihood of therapeutic efficiency and to lessen the risk of drug toxicity for an individual patient. Pharamcogenomic analysis can reduce adverse drug reactions (ADRs) which occur approximately in 2 million people each year and causes as many as 100,000 deaths according to the Food and Drug Administration (FDA) [Adams, 2008]. The main goal of pharmacogenomics research is predicating and pre-emptive ADRs [Adams, 2008]. Large number of clinical trials and recent findings in pharamcogenomics have shown that the genetic variations in genes for drug metabolizing enzymes, drug receptors, and drug transporters can alter drug metabolization and cause variable drug responses in individual patients [Roden and George, 2002]. Currently more than 100 drugs have labeled information regarding pharmacogenomic biomarkers.

Warfarin therapy is one of the many clinical situations in which knowledge of pharmacogenomics is helping in minimizing ADRs. Warfarin has shown 20-fold difference in the dosage of warfarin required to achieve the desired therapeutic effect when given to different patients [Adams, 2008]. Though for more than 6 decades, this therapy is in routine practice for various clinical conditions, the complications associated with this therapy are still not completely resolved. Several studies in India [Patel et al., 2007; Pattanaik et al., 2009] and the world [Zaidenstein et al., 2002; Pirmohamed et al., 2004; Landefeld and Beyth, 1993] have reported that anticoagulation induced bleeding is one of the top adverse drug reactions, associated with hospitalization. Large scale randomized clinical trials are in progress to develop a globally applicable dosing strategy for warfarin. Various genetic and non genetic factors have been found to be associated with warfarin response. However, the contribution of genetic factors has been shown to be quite significant. The genotypes of Vitamin K epoxide reductase complex subunit 1 (VKORC1) and

Tejasvita Gaikwad
Senior Research Fellow
Department of Haemostasis & Thrombosis, National Institute of Immunohematology (ICMR). 13th Floor, New Multistoried Building, KEM Hospital Campus, Parel, Mumbai 400 012. Email: gtejasvita@gmail.com

Cytochrome P450-2C9 (CYP2C9) have been found major contributors of warfarin response; genetic variations in Cytochrome P450-4F2 (CYP4F2), Gamma-glutamyl carboxylase (GGCX), Epoxide hydrolase 1 (EPHX1), Calumenin (CALU), Factor IX (F9) and Factor VII (F7) genes also have shown subsidiary association with warfarin response. Other non-genetic factors including ethnicity, age, body mass index (BMI), weight and gender are also known to have some effect on warfarin response.

Mode of action of warfarin:

Warfarin's key mode of action is inhibiting the synthesis of vitamin K-dependent clotting factors, which include Factors II (F2), VII (F7), IX (F9), and X (F10), and the anticoagulant proteins C and S **(Fig.1).**

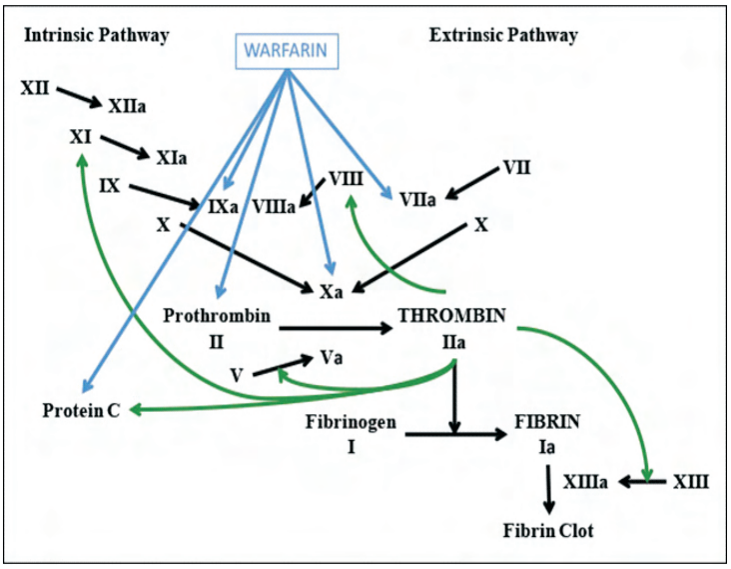

Fig. 1: Coagulation cascade showing points of activity of warfarin (a vitamin K antagonist)
[Adapted from Davis et al., 2013]

Warfarin is thought to interfere with the cyclic inter-conversion of vitamin K and its vitamin K epoxide (2,3 epoxide). Vitamin K-dependent proteins, which include the coagulation factors II (*F2*), VII (*F7*), IX (*F9*), and X (*F10*) along with protein C, protein S and protein Z, require γ-carboxylation by vitamin K for biological activity. Vitamin K acts as a cofactor for the carboxylation of glutamate residues to γ-carboxyglutamates (Gla) on the N-terminal regions of vitamin K–dependent proteins (**Fig. 2**) [Whitlon et al., 1978; Fasco et al., 1982; Choonara et al., 1988; Trivedi et al., 1988; Stenflo et al., 1974; Nelsestuen et al., 1974; Hirsh et al., 2003]. By inhibiting the vitamin K conversion cycle, warfarin induces hepatic production of partially decarboxylated proteins with reduced coagulant activity [Friedman et al., 1977; Malhotra et al., 1985; Hirsh et al., 2003]. γ-Carboxylation requires the reduced form of vitamin K (vitamin KH2) and warfarin blocks the formation of vitamin KH2 by inhibiting the enzyme vitamin K epoxide reductase, thereby limiting the γ-carboxylation of the vitamin K–dependent coagulant proteins.

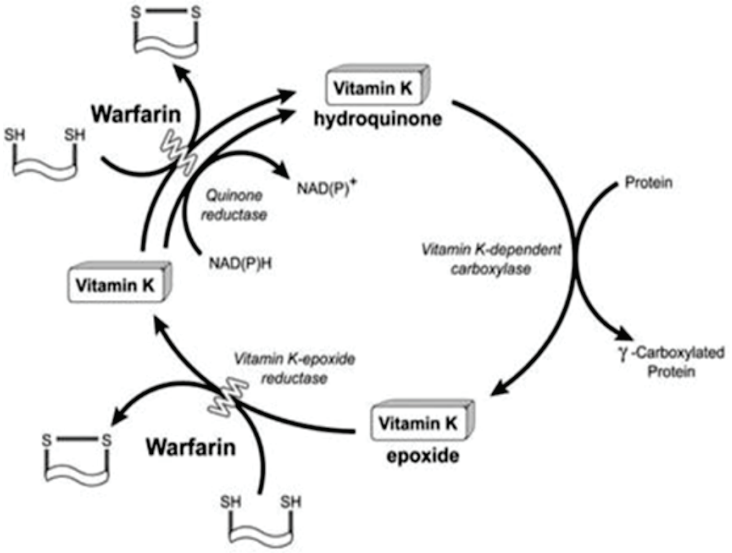

Fig. 2: Mode of action of warfarin
[Adapted from: Higdon, 2000]

Indications and duration of therapy:

Warfarin has been used to decrease the tendency for thrombosis or as secondary prophylaxis i.e. prevention of further episodes. **Table 1.** shows various clinical conditions and their respective recommended INR range and duration of therapy.

Table 1. Indications and therapy details (Table adapted from Horton et al., 1999)

INDICATION	INR	DURATION
Prophylaxis of venous thrombosis for high-risk surgery	2 to 3	Clinical judgment
Treatment of venous thrombosis		
First episode	2 to 3	3 to 6 months
High risk of recurrent	2 to 3	Lifelong
Thrombosis associated with antiphospholipid antibody	3 to 4	Lifelong
Treatment of pulmonary embolism		
First episode	2 to 3	3 to 6 months
High risk of recurrent embolism	2 to 3	Lifelong
Prevention of systemic embolism		
Tissue heart valves	2 to 3	3 months
Acute myocardial infarction (to prevent systemic embolism)†	2 to 3	Clinical judgment
Valvular heart disease (after thrombotic event or if the left atrium is greater than 5.5 cm)	2 to 3	Lifelong
Atrial fibrillation		
Chronic or intermittent	2 to 3	Lifelong
Cardioversion	2 to 3	3 weeks before and 4 weeks after atrial fibrillation if normal sinus rhythm is maintained
Prosthetic heart valves		
Aortic position Mechanical	2.5 to 3.5‡	Lifelong
Bioprosthetic	2 to 3	Clinical judgment (3 months optional)
Mitral position		
Mechanical	2.5 to 3.5‡	Lifelong
Bioprosthetic	2 to 3	3 months

INR = International Normalized Ratio.

†—If oral anticoagulant theraphy is elected to prevent recurrent myocardial infarction, an INR of 2.5 to 3.5 is recommended.

‡—Depending on the type of mechanical valve (i.e., caged ball or caged disk) and the valve position (mitral), some patients may benefit from INRs in the upper end of the range.

Factors affecting warfarin response

Warfarin is one of the top 10 drugs related to adverse drug events and hospitalization. Clinical management of warfarin therapy is mainly complicated by its narrow therapeutic index and effect of various genetic, demographic, clinical and environmental factors on warfarin response (**Fig. 3**).

Fig. 3: Factors affecting warfarin response

Warfarin Pharmacogenetics:

A) Pharmacokinetics: *CYP2C9*

Absorption:

Warfarin is rapidly and completely absorbed from the stomach and proximal small intestine with peak blood concentrations within 0.3 to 4 hours [Pyoralak et al., 1971]. Warfarin is nearly 100% bioavailable.

Distribution:

Warfarin gets distributed into a relatively small apparent volume of distribution of about 0.14 L/kg. A distribution phase lasts for 6 to 12 hours. It circulates bound to plasma proteins mainly albumin and gets accumulated in the liver, where the two isomers are metabolically transformed by different pathways [O'Reilly et al., 1987].

Metabolism:

Warfarin is extensively metabolized via Cytochrome P450 system. S-warfarin is approximately 90% oxidized, primarily by *CYP2C9* to S-6-hydroxywarfarin and S-7-hydroxywarfarin is formed in a 3:1 ratio, and to a lesser extent by *CYP3A4* (Cytochrome P450 3A4) to S-4'-hydroxywarfarin and S-10-hdyroxywarfarin [Wittkowsky, 2011]. R-warfarin is approximately 60% oxidized by *CYP1A2* (Cytochrome P450, family 1, subfamily A, polypeptide 2) to R-6-hydroxywarfarin and R-7-hydroxywarfarin, by *CYP3A4* to R-10-hydroxywarfarin and R-4'-hydroxywarfarin, and by *CYP2C19* (Cytochrome P450 2C19) to R-8-hydroxywarfarin [Wittkowsky, 2011] (**Fig. 4**).

Elimination:

Inactive oxidative metabolites and reduced alcohol derivatives of warfarin are eliminated by urinary excretion [Toon et al., 1986].

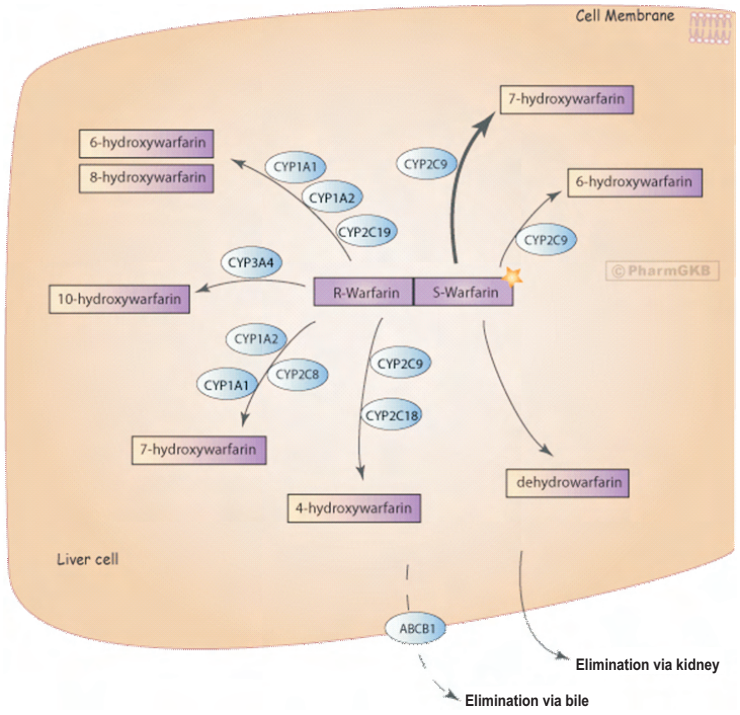

Fig. 4: Representation of the candidate genes involved in transport, metabolism and clearance of warfarin (Pharmacokinetics) (Adapted from www.pharmgkb.com)

Warfarin pharmacokinetics in association with ***CYP2C9*** is considered as a classical example of pharmacogenetics. *CYP2C9* is a hepatic drug-metabolizing enzyme in the *CYP450* superfamily and is the primary metabolizing enzyme of S-warfarin [Lee et al., 2002]. The two most important variants shown to have clinical implications for warfarin dosing are *CYP2C9**2 and *CYP2C9**3. Individuals homozygous for the reference *CYP2C9* allele (*CYP2C9**1) have the "normal metabolizer" phenotype. In vitro and ex vivo studies suggest that *CYP2C9**2 and *3 impair metabolism of S-warfarin by ~30–40% and ~80–90%, respectively [Lee et al., 2002]. Individuals inheriting the *2 and *3 variants need lower doses of warfarin (**Table. 2** and **Fig. 5**), take a longer time to reach target INR during initial phase of warfarin therapy and have an increased risk of bleeding complications [Aithal et al., 1999; Higashi et al., 2002].

Table 2: CYP2C9 variants and their relationship to warfarin metabolism (Adapted from McClain et al., 2008)

CYP2C9		
Genotype	Metabolism	Nomenclature
*1/*1	Extensive, rapid, ultra-metabolizer	Normal, wild
*1/*2	Intermediate	Heterozygote
*1/*3	Poor, slow	Heterozygote
*2/*3	Poor, slow	Compound heterozygote
*2/*2	Poor, slow	Homozygote
*3/*3	Extremely slow	Homozygote

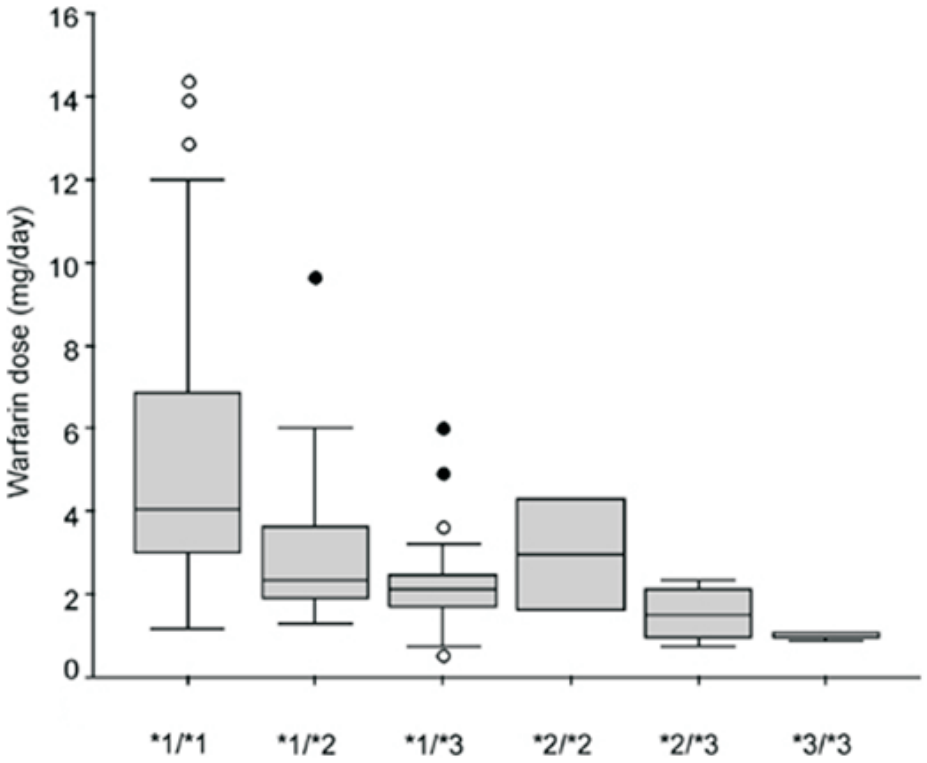

Fig: 5. The influence of CYP2C9 genotype on warfarin maintenance dose (Adapted from Herman et al., 2005)

It is also well known that, the frequencies of the *CYP2C9* variant alleles differ between racial/ethnic groups [Limdi et al., 2010; Lee et al., 2002; Wu et al., 2008; Sanderson et al., 2005; Xie et al., 2002].

B) Pharmacodynamics: *VKORC1*

The Warfarin pharmacodynamics pathway depicts vitamin K epoxide reductase and the downstream genes whose products are postranslationally carboxylated to become Gla-containing proteins by gamma-glutamyl carboxylase. These Gla-containing proteins take active part in maintaining hemostasis (coagulation factors FII,FVII,FIX, FX, Protein C, S and Z) (**Fig.6**).

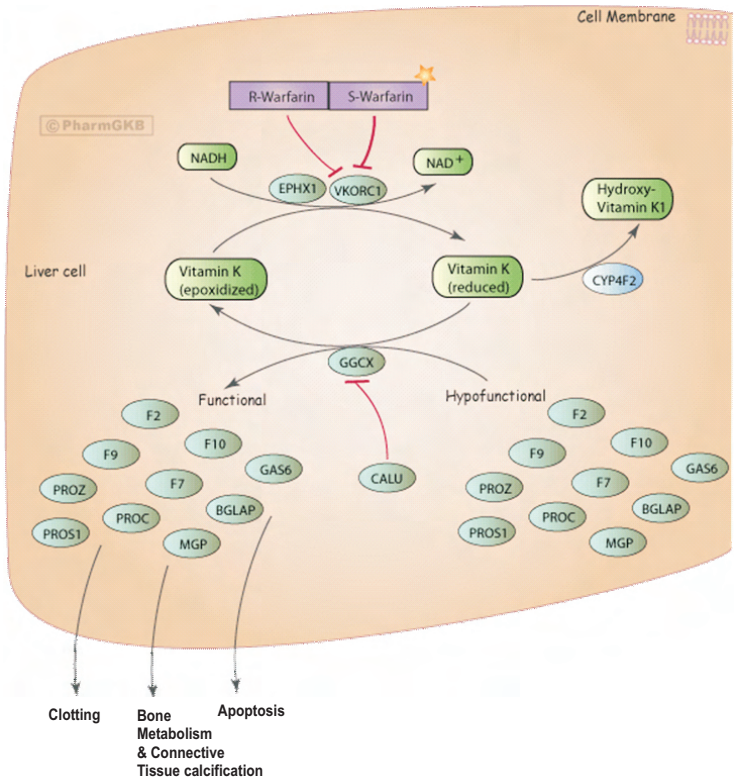

Fig. 6: Simplified diagram of the target of warfarin action and downstream genes and effects
(Adapted from www.pharmgkb.com)

So far several variants in *VKORC1* have been reported to influence warfarin pharmacodynamics. Among these variants, *VKORC1* -1639 G>A has shown stronger influence to alter warfarin pharmacodynamics. **Table. 3** and **Fig. 7** show, the effect of *VKORC1* -1639G>A variant on enzyme activity and dose requirement.

Table 3. VKORC1 -1639 G>A enzyme activity

***VKORC1*-1639G>A**	
Genotype	**Enzyme activity**
GG (Wild type)	Normal enzyme activity
GA (Heterozygous variant)	Intermediate enzyme activity
AA (Homozygous variant)	Low enzyme activity

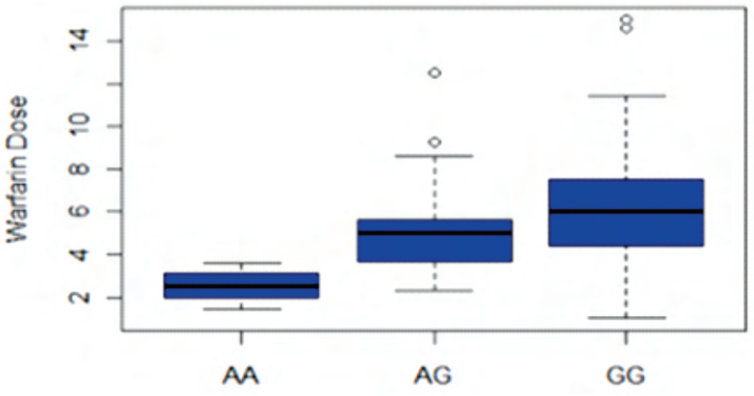

Fig. 7: Warfarin dose vs VKORC1 genotype
(Adapted from http://web.stanford.edu/)

C) Others

Growing evidence indicates that maximum inter-individual variation in response to warfarin might be due to genetic variables and affected by polymorphisms in the genes mainly, Vitamin K epoxide reductase complex subunit 1 (*VKORC1*) and cytochrome P450-2C9 (*CYP2C9*). Although the genotypes *VKORC1* and *CYP2C9* are clearly the most important genetic factors for warfarin response [Rieder et al., 2005; Geisen et al., 2005; Sullivan-Klose et al., 1996], association has also been found with genetic variations in other genes which include *CYP4F2, GGCX, EPHX1, CALU*, factor IX (*F9*) and factor VII (*F7)* (**Fig. 8**).

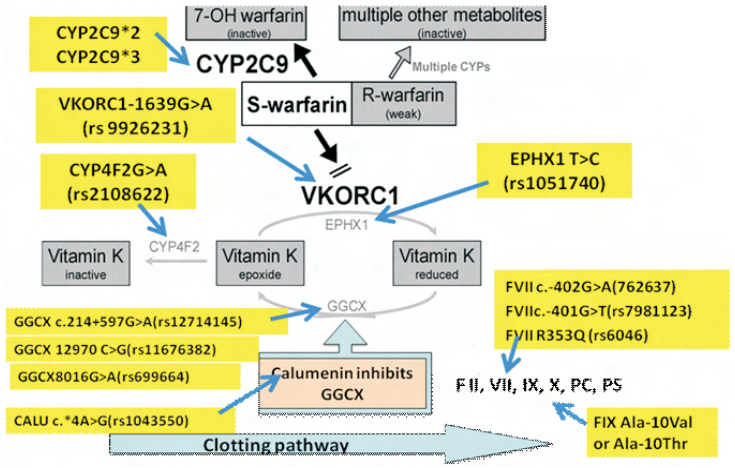

Fig. 8: Genetic variants [highlighted in yellow] known to be associated with change in warfarin action

CYP4F2 :

CYP4F2 variants have been found to be associated with warfarin dose and are speculatively linked to altered vitamin K1 metabolism. *CYP4F2* is a vitamin K1 oxidase and carriers of the *CYP4F2* V433M (rs2108622) allele have shown a reduced capacity to metabolize vitamin K1, secondary to an rs2108622-dependent decrease in steady-state hepatic concentrations of the enzyme. Therefore, patients with the rs2108622 polymorphism are likely to have elevated hepatic levels of vitamin K1, and require a higher warfarin dose to obtain the same anticoagulant response [McDonald et al., 2009; Deng et al., 2010].

GGCX:

Gamma-glutamyl carboxylase encoded by *GGCX* uses reduced vitamin K to activate clotting factors II (*F2*), VII (*F7*), IX (*F9*) and X (*F10*) and proteins C, S and Z. In the process, vitamin K is oxidized, and in the next cycle VKOR regenerates reduced vitamin K. Warfarin inhibits VKOR, impairing the synthesis of clotting factors. [Wadelius et al., 2005]. Several variants in *GGCX* gene are found to be linked to altered warfarin response; however few other reports do not show such an association [Wadelius et al., 2005; Shikata et al., 2004; Cavallari et al., 2012; King et al., 2010; Luxembourg et al., 2011].

***EPHX1*:**

Epoxide hydrolase 1 gene (*EPHX1*) encodes the enzyme microsomal epoxide hydrolase 1(mEH) enzyme. This enzyme carries a vitamin K 2, 3 -epoxide binding site and genetic variants of this enzyme are believed to alter warfarin metabolism causing changes in the vitamin K cycle, resulting in altered warfarin pharmacodynamics [Pautas et al., 2010; Chan et al., 2011; Carlquist et al., 2010]. Two association studies show a probable role for *EPHX1* in varying warfarin dose requirement, though this remains to be confirmed by biochemical experiments [Wadelius et al., 2005; Loebstein et al., 2005].

Calumenin:

Warfarin exerts its anticoagulant effects by inhibiting vitamin K epoxide reductase (VKOR) and prevents reduction of vitamin K, which is necessary for γ-carboxylation of clotting factors II, VII, IX , and X . Both γ-glutamyl carboxylase and calumenin are involved in the γ-carboxylation of clotting factors. Few studies have reported minor impact of Calumenin variants on warfarin dose requirement [Glurich et al., 2013; Vecsler et al., 2006; González-Conejero et al., 2007].

Factor VII(*F7*) and Factor IX(*F9*) gene polymorphisms:

Genetic variation within the genes coding for vitamin K dependent proteins have been suggested to predict sensitivity to warfarin therapy. Few studies have reported, effect of factor VII (*F7)* and IX (*F9*) genetic variants on warfarin response [D'Ambrosio et al., 2004; Aquilante et al., 2006; Shikata et al., 2004]. However impact of these variants is very subtle on the overall warfarin response [Mlynarsky et al., 2004; Mlynarsky et al., 2012].

NIIH Experience:

Three Hundred patients who were prescribed warfarin for various clinical conditions were analysed to study the effect of genetic and non-genetic determinants on warfarin dosage and other associated adverse conditions associated with the drug. The objective of this study was to draw a pharmacogenetic algorithm based on comprehensive analysis of warfarin linked polymorphisms and non genetic factors for an appropriate personalized warfarin dosage which will help in minimizing warfarin induced adverse effects. Following a literature review, 21 genetic and non genetic factors were selected for investigation. Patients with abnormal liver/ renal function, needing a medication known to interact with warfarin and patients with severe diseases known to interfere with this treatment were excluded from this study. Around 44% Indian patients carry two strongly associated warfarin sensitive alleles i.e. VKORC1 -1639 A and CYP2C9 *2/ *3. About 9% Indian patients face severe bleeding manifestation during warfarin therapy [Gaikwad et al., 2013]. Genetic factors: Among the 16 polymorphisms analyzed, only 5 showed significant association with warfarin response. Important conclusions of this study are as follows:

Four tag single-nucleotide polymorphisms (SNPs) (861C>A, 5808T>G, 6853G>C, and 9041G>A) were found to be in linkage disequilibrium (LD) with VKORC1-1639G>A (3673G>A) polymorphism and both these are good predictors of the warfarin dose requirement. The haplotype analysis does not add more information than the single SNP analysis i.e. VKORC1-1639G>A (3673G>A)

The polymorphisms which were found to be associated with warfarin dose variability and risk of overanticoagulation are VKORC1 -1639 G>A, CYP2C9 genotype, CYP4F2 G>A (rs2108622), EPHX1 T113C (rs1051740) and CALU c.*4A>G (rs1043550). Hence these polymorphisms will be beneficial to formulate warfarin pharmacogenetic algorithm in Indian context. Besides genetic factors, age and diet were found to be an important clinical parameter for fixing the daily warfarin dose and also predict the risk of overanticoagulation.

Skipping of four VKORC1 haplotype tag SNP's, GGCX c.214+597G>A, GGCX 12970 C>G, GGCX 8016 G>A, two factor VII promoter region polymorphisms (i.e. c.402G>A & c.401G>T) and FVII RR353Q can make warfarin pharmacogenetics more cost effective in Indian population.

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NIIH HAPPENINGS

Department of Pediatric Immunology & Leukocyte Biology

Dr Manisha Madkaikar, Scientist F

Invited to deliver a lecture on MSMD in the symposium entitled "Life At War: Treading the War Zone" organized by Division of Immunology, Bai Jerbai Wadia Hospital for Children, Mumbai on 19th July 2015.

Manasi Kulkarni, SRF

Received Student Travel Award for attending 30th Annual meeting of ISAC, held at Glasgow, Scotland, United Kingdom from 26th-30th June, 2015 and presented a poster entitled "Flowcytometry as rapid diagnostic tool for molecular screening of chronic granulomatous disease"

Department of Hematogenetics

Dr Malay Mukherjee, Scientist E

Visited Bulsar Raktadan Kendra, Valsad and Seva Rural Hospital, Jagadiha, Baruch in relation to New Born Screening programme among the tribal groups of South Gujarat from 19th to 20th May 2015.

Invited to participate in the Workshop entitled "Sickle Cell Anemia and Water Bodies" organized by the Tribal Research Institute, Government of Karnataka, held at Mysore on 31st May 2015 and delivered a lecture on "Hemoglobinopathies in India".

Invited to participate in the Workshop entitled "Sickle Cell Anemia and Water Bodies" organized by the Tribal Research Institute, Government of Rajasthan, held at Udaipur on 13th June 2015 and delivered a lecture on "Hemoglobinopathies in India".

Invited to participate in the Workshop entitled "Sickle Cell Anemia and Water Bodies" organized by the Tribal Research Institute, Government of Maharashtra, held at Pune on 20th June 2015 and delivered a lecture on "Hemoglobinopathies in India".

Visited Sickle Cell Satellite Centre, Chandrapur on 27th June 2015 to supervise the renovation work carrying out over there.

Invited to participate in the Workshop entitled "Sickle Cell Anemia and Water Bodies" organized by the TCR&TI, Government of Telengana, held at Hyderabad on 11th July 2015 and delivered a lecture on "Hemoglobinopathies in India".

Attended Tribal Health Research Unit Review meeting held at NIE, Chennai on 16th July 2015 and presented the work done at our centre.

Attended IITB Healthcare Consortium Management Committee Meeting held at IIT, Mumbai on 24th July 2015.

Convener for Master Training Programme on Sickle cell Anemia, organized by TRTI, Government of Maharashtra, held at Pune on 4th August 2015.

Attended "Tribal Health Research Forum Meeting" held at NIRTH, Jabalpur from 8th to 9th August 2015 and presented the work done at NIIH, Mumbai.

Attended Expert Group Meeting for Control of Sickle Cell disease held at Arogya Bhavan, Mumbai on 29th August 2015.

Department of Transfusion Medicine

Dr Ajit Gorakshakar, Scientist F

Awarded best Poster on the paper entitled "Development of surface anchored silver nanoparticles: A novel and easy blood group detection system based on nanotechnology" in the Biomaterials International conference held at Kenting, Taiwan from 1-5 June 2015.

Attended expert committee meeting on "RDB kit" at ICMR headquarters on 5th June 2015.

Department of Cytogenetics

Dr V Babu Rao, Scientist E

Attended executive committee meeting of Molecular Pathologists Association of India on 8th June 2015.

Invited as Chairman of selection committee for the selection of candidates for JRF, SRF and Project assistant at NIRRH, Mumbai on 16th July 2015.

Invited to deliver a lecture for Ph.D students on "Advances in Genetics" at NIRRH, Mumbai on 18th August 2015.

Attended as an external expert for the selection of candidates for Ph.D courses at NIRRH, Mumbai on 25th and 26th August 2015.

Fairwell to
Mr. C.T. Kulkarni,
Technical Officer

Fairwell to
Dr. Roshan B. Colah,
Director Incharge

Nisargopchar Meet

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