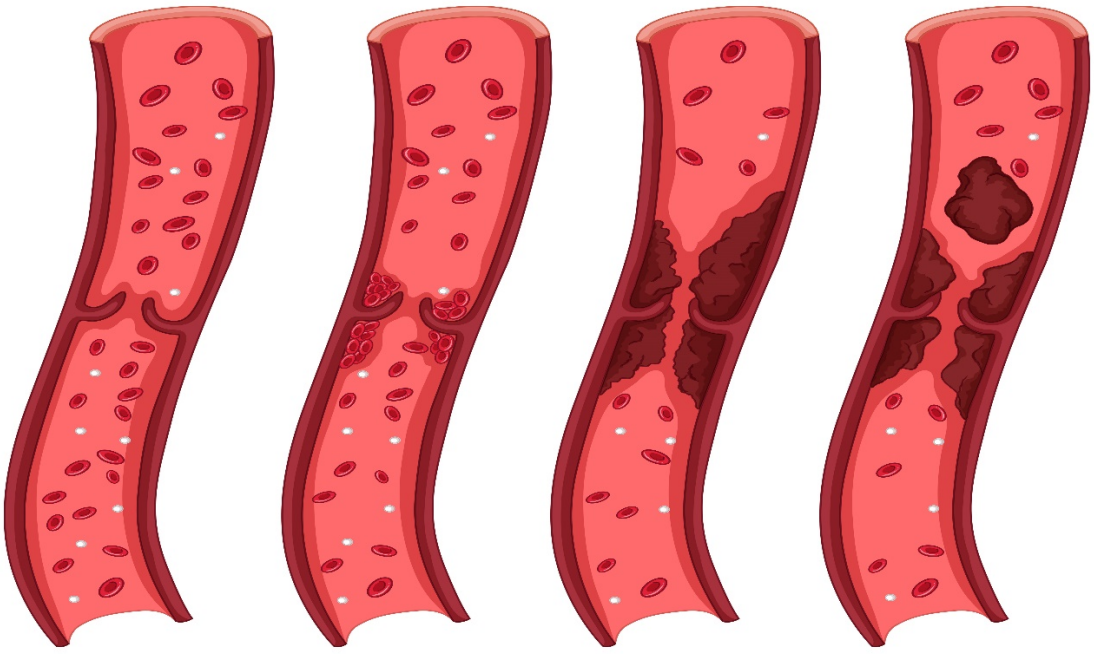
DNK s.r.o.
diagnostické centrum

Laboratory diagnosis of genes affecting warfarin metabolism

Diagnostic kits to detect polymorphisms affecting warfarin dosage and identify patients at risk



Features of warfarin treatment

Arterial and venous thromboses are the leading cause of life-threatening complications in patients with cardiovascular disease. One of the drugs for the treatment and prevention of thromboembolic complications is **warfarin** (WF).

Warfarin belongs to the group of vitamin K antagonists, also called indirect anticoagulants. The antithrombotic effect of the drug lies in the ability to block the synthesis of blood coagulation factors dependent on vitamin K (II, VII, IX, X).

Currently, warfarin has no analogues in the treatment of patients with prosthetic heart valves and atrial fibrillation.

Warfarin dose variability reaches 30-fold differences

The disadvantage of antithrombotic therapy with warfarin is an increased risk of bleeding. The variability in the level of anticoagulation depends on many factors and determines the need for individual selection of the dose of warfarin.

The generally accepted method of monitoring the effectiveness and safety of warfarin therapy is an **international normalized ratio (INR)**, which is calculated on the basis of the patient's prothrombin test result. On average, to achieve effective anticoagulant therapy without the risk of complications, it is recommended to maintain an INR value in the range of 2.0-3.0 in people treated with warfarin.

It takes a long time to achieve and maintain a narrow therapeutic range of INR. At the stage of selecting the optimal dose of warfarin, the risk of bleeding remains in patients with high sensitivity to the active substance or risk of recurrent thromboembolic complications in patients with resistance to warfarin.

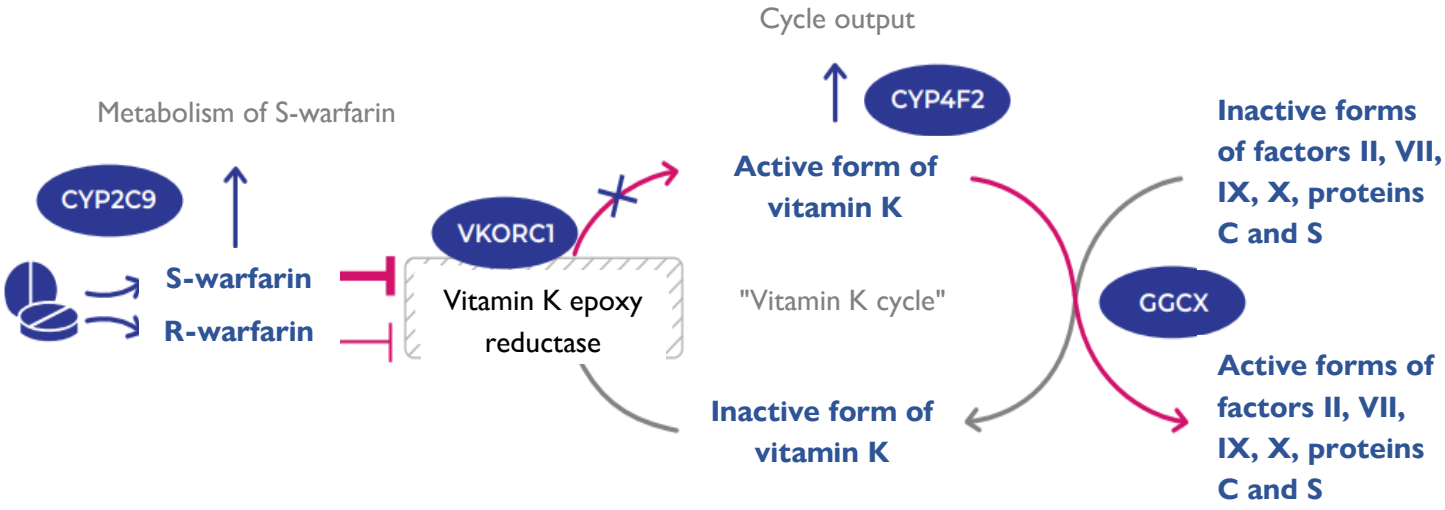
< 15%	0,4- 7,2%	4,5-16%
frequency of heavy bleeding per year	cases of minor bleeding per year	the risk of retrombosis during warfarin therapy per year

The variability of the dose of warfarin is determined not only by clinical factors (weight, gender, smoking, etc.), but also by genetic factors. Of particular importance are allelic variants of genes involved in the main stages of warfarin pharmacokinetics.

53-54%	17-21%
contribution of genetic factors	contribution of clinical factors

Contribution of polymorphisms of the génov CYP2C9, VKORC1, CYP4F2 and GGCX genes to warfarin dose variability

Warfarin is a mixture of R- and S-isomers, the action of which is aimed at inhibiting the enzyme vitamin K epoxy reductase, which is involved in the activation of vitamin K. The active form of vitamin K starts the process of "maturation" of blood coagulation factors (II, V, VII, X), as well as anticoagulant proteins C and S.



The most significant genes, in which polymorphisms determine the individual response to warfarin therapy:

CYP2C9 – encodes for an enzyme involved in the main pathway of biotransformation of the warfarin S-isomer. S isomer is 5 times more effective than R isomer and therefore has greater clinical significance.

VKORC1 – encodes the enzyme Vitamin K, epoxy reductase. Gene polymorphisms determine the variability of warfarin doses in 25-30% of cases.

CYP4F2 – a gene product that controls excessive accumulation of vitamin K.

GGCX – gene product involved in the activation of blood coagulation factors (II, V, VII, X) and anticoagulant proteins C and S.

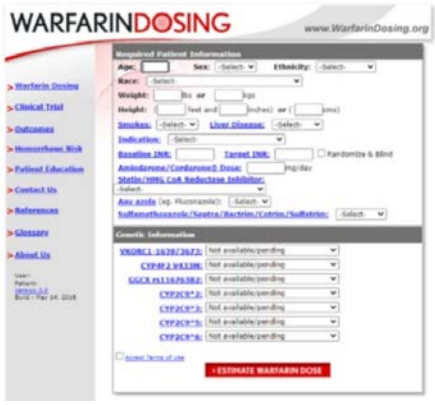
Features and advantages of the diagnostic kit

- **Ready Master Mix for PCR**: Simplification of analysis procedures and high stability of test quality
- **Specimens**: whole blood or buccal epithelium
- **Number of tests**: kits are designed for the analysis of 48 samples including control samples
- **Multiplexes:** detection of more markers in a single tube
- **Compatible devices**: CFX96 (Bio-Rad, USA), DT-96 a DTprime (DNA-Technology, Russia), Gentier 96E/R (Xi'an TianLong, Science and Technology Co., Ltd., China)
- **High stability of the kit:** storage at a temperature of 2–8 ° C; transport up to 26 °C not more than 10 days

Influence of genotype on warfarin dosage

CYP2C9	CYP2C9*2 CYP2C9*3	Among Europeans, the frequency of the allele 2* is 8%, the allele 3* 4%	Decreased rate of biotransformation of warfarin, high sensitivity to the drug
	CYP2C9*5 CYP2C9*6	The carrier is characteristic of the Negroid race	
VKORC1	Haplotype A (1173 S/T)	Among Caucasians, the frequency is 35%, among Asians 80%	Decreased expression of enzymes, high sensitivity to warfarin
	Haplotype B (1173 S/T)	The carrier is characteristic of the Negroid race	Increased expression of enzymes, increased resistance to warfarin
CYP4F2	CYP4F2*3 (1297 C/T)	Among Caucasians, the frequency is 23%, among Asians, 26%, among the black race 8%	Increase in vitamin K, increased resistance to warfarin
GGCX	Allele G (rs11676382 C/G)	The carrier is characteristic of the Eurasian race	Lack of coagulation factors, high sensitivity to warfarin

The pharmacogenetic approach is most effective in choosing the dose of warfarin



The calculation of an individual dose of warfarin based on the results of molecular-genetic analysis can be carried out, for example, using an international source www.WarfarinDosing.org.

The calculation algorithm is based on the evaluation of clinical factors (age, weight, gender, race, lifestyle (smoking), liver disease, diet, drugs that modulate the action of warfarin) and genetic factors (genotypes CYP2C9, VKORC1, CYP4F2 and GGCX).

According to clinical recommendations "Prevention of thromboembolic syndromes"

Genetic polymorphisms of the biotransformation gene (CYP2C9) and target molecules (VKORC1) significantly influence the pharmacodynamic effects of warfarin. Pharmacogenetic testing is recommended for personalised selection of the dosing regimen for indirect anticoagulants.

Diagnostic kits for the detection of genetic polymorphisms associated with dose variability of warfarin by PCR with melting curve detection

Cat. №	Kit name	Number of tests
	Extraction kits for the isolation of nucleic acids	
8845	**RealBest-Genetics DNA-express**	50
8846	**RealBest GenMag**	96 (2x48)
	SNP detection kits associated with warfarin dose variability	
3827 CE	**RealBest-Genetics Warfarin (CYP2C9*2/CYP2C9*3)**	48
3828 CE	**RealBest-Genetics Warfarin (VKORC1/CYP4F2*3)**	48
3829 CE	**RealBest-Genetics Warfarin (GGCX)**	48
3830 CE	**RealBest-Genetics Warfarin (CYP2C9*5/CYP2C9*6)**	48

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