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Bioinformatics-based of warfarin individualized medication research in cardiac surgery patients

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Abstract

Warfarin is an oral anticoagulant that is widely prescribed worldwide, and it has a large individual variability. Many factors contribute to the variability of warfarin. Although numerous algorithms of warfarin have also been developed by pharmacokinetics and pharmacodynamics (PKPD) and multivariate linear regression (MLR) models, there also exits some unknown factors that could affect the warfarin anticoagulant. In this study, the precision medication model of warfarin will be established based bioinformatics in cardiac surgery patients. About 200 cardiac surgery patients who will be administered warfarin will be enrolled. The demographic characters, combined drug and physiological factors will be collected from patients' medical records. Some of single nucleotide polymorphisins (SNP) linked with warfarin PK and PD for pharmacogenetics will be performed using pyrosequencing. Fecal samples will be used for analyzing gut microbiota by 16S rDNA. S-warfarin, R-warfarin and vitamin K concentrations, and metabolomics will be detected by LC-MS/MS. International normalized ratio (INR) is the effective efficacy index for warfarin, and target INR should be within 1.5-2.5. All data will be analyzed by machine learning or neural networks. Expected the polymorphisms of pharmacogenomics (CYP2C9, VKORC1, and CYP4F2 etc.), vitamin K concentrations, and other biomarkers from gut microbiota and metabolomics were confirmed as the effect factor for the individual variability of warfarin. And an artificial intelligence model of warfarin would be established.

Introduction *(optional)*

Warfarin is an oral anticoagulant that is widely prescribed worldwide [1-3]. Although novel oral anticoagulants (dabigatran, rivaroxaban, apixaban, and edoxaban), that have a lower risk of serious bleeding complications than warfarin in prevention and treatment for atrial fibrillation (AF), deep vein thrombosis (DVT) and pulmonary embolism (PEm) patients [4-6] have appeared in the marketplace, the novel anticoagulants are more expensive, and one of the studies that compared the effect between dabigatran and warfarin showed that dabigatran had a worse effect than warfarin in patients with mechanical heart valves[5]; therefore, warfarin remains the mainstay of anticoagulant therapy[7, 8].

Warfarin has a large individual variability and a narrow therapeutic index, and the daily dose variability may be 5- to 20-fold when achieving an anticoagulant effect [9]. Many factors contribute to the variability of warfarin. In addition, numerous algorithms of warfarin have also been developed by pharmacokinetics and pharmacodynamics (PKPD) [10, 11] and multivariate linear regression (MLR) models [3, 12]. There also exits some unknown factors that could affect the warfarin anticoagulant.

The chemical structure of warfarin is similar to vitamin K. Warfarin exerts its anticoagulation by interfering with the synthesis of vitamin K dependent clotting factors via inhibition of vitamin K epoxide reductase complex 1 (VKORC1) [7]. Vitamin K includes two major forms: phylloquinone (vitamin K1) and menaquinones (vitamin K2, MK4-MK13). Phylloquinone (vitamin K1) is found exclusively in plants, while menaquinone (MK5-MK13) is produced by a series of congeners synthesized by gram-positive bacteria in the human gastrointestinal tract [13, 14], MK4 is endogenously synthesized from phylloquinone in mammals [15] and is found in animal products. All forms of vitamin K have one well-known function which were identified as a cofactor for the post-translational enzyme γ-glutamate carboxylase, which is established by the common naphthoquinone ring structure. It is necessary for the generation of active coagulation factors II, VII, IX, and X [13, 14]. The liver is the site of synthesis of the vitamin K-dependent coagulation factors and was originally thought to be the major site of storage of vitamin K [15]. So, the fluctuations in vitamin K concentration in the body maybe affect the warfarin anticoagulant effects.

The information on the physiological and pharmacological roles of vitamin K in vivo is still limited. One reason for this is that the detection and monitoring of vitamin K homologues in plasma and organs have been difficult on account of quite small concentrations and many kinds of impurities even though measurement of the vitamin K plasma concentration is essential to optimize therapy [16]. The detection of vitamin K1 had been developed in plasma, however, the detection of vitamin K2 is difficult in plasma. The human gut is thought to be a menaquinone reservoir and is estimated to contribute to 10–50% of the human vitamin K requirement [14,17]. And some studies indicated that phylloquinone accounted for less than 10% of the vitamin K content in the human liver [14]. Vitamin K2 in vivo could be reflected by analyzing the gut microbiota composition. The major microbial phyla that represent over 90% of the bacterial component of gut microbiota are *Firmicutes*, *Bacteroides*, *Proteobacteria* and *Actinobacteria* [18].

Gut microbiota is characterized by an inter-individual variability due to genetic and environmental factors [19]. Many factors, either exogenous or endogenous, affect the composition of the gut microbiota. These factors include host genotype, age, and sex [20]. However, of all the environmental factors studied to date, lifestyle (such as dietary habits), one of the enviromental factors, play an improtmant role in the modulation of gut microbiota [18-21], and the use of antimicrobial drug also perturb the composition of the microbiota [21]. For most of the dominant genera in the human gut microbiota, inter-individual variations were significantly larger than intra-individual variations [19]. The human gut microbiota is seeded during birth and mainly develops over the first 3 years of life. Over the first 3 years of life, the composition of the microbial community becomes more adult-like, and major microbial shifts are associated with key events such as the introduction of solid food [20]. Gut microbiota was found an apparently smooth change in the first 3 years of life, followed by relatively subtle changes in cross-sectional studies [20]. The subject-specific microbiota is thought to be stable for a long period, whereas the composition of each human intestinal bacterium within the microbiota has been demonstrated to change rapidly with a diet rich in fermented milk [19].

Quantitatively, the most important genera of intestinal flora are the *bacteroides* and *bifidobacteria*, which together can account for over half of the total anaerobic bacterial population; they differ, however, in that only the *bacteroides* synthesize menaquinones [15]. Bacterium that synthesizes vitamin K2 in the intestine includes: *Bacteroides*(MK6 and MK8–MK13), *Proteobacteria* (MK9–MK10), *Firmicutes* (MK9–MK10), *Actinobacteria*(MK5–MK7/MK11–M13), *Prevotella*(MK5–MK9/MK11–M13), *Ruminococcaceae*(MK6 and MK8–MK13), *Alistipe*(MK6 and MK8–MK13), *Oscillibacter*(MK6 and MK8–MK13), *Bilophila*(MK6 and MK8–MK13), *Odoribacter*(MK6 and MK8–MK13), *Barnesiella* (MK6 and MK8–MK13), *Escherichia* (MK8), *Shigella* (MK8), *Klebsiella* (MK8) [17, 22], *Eubacterium lentum* (MK6), *Lactococcus lactis ssp. Lactis* and *L. lactis ssp. Cremoris* (MK8 and MK9), *propionibacteria* (MK9) [14]. So, it postulate that vitamin K is an important factor in warfarin anticoagulation.

The hypothesis of this study is the anticoagulation of warfarin would be affected by the vitamin K fluctuations in body. The objectives of this study are to explore the factors that influence the fluctuation of vitamin K in vivo, further explore the factors that affect the anticoagulant efficacy of warfarin, and to establish an artificial intelligence model of warfarin.

Materials and Methods *(optional)*

Patients and sampling

This study will be conducted in the First Affiliated Hospital of Soochow University, Suzhou, China. All patients will be administrated warfarin because patients will be operated with cardiac surgery. The number of the patients is about 200 patients. All patients will be followed up during hospital stay.

CYP 2C9 and VKORC1 are performed as part of standard clinical care. And other gene polymorphisms (such as CYP4F2, microRNAs) associated with warfarin metabolism and action also will be genotyped.

The routine detection of international normalized ratio (INR) are performed in hospital clinical laboratory. About 3ml blood will be drawn in a coagulation tube when drawing blood for INR detection. These blood sampling will be centrifuged, then serum samples will be restored in -80 ℃. The serum samples will be used for detecting S-warfarin, R-warfarin and vitamin K1concentrations, and metabolomics.

Fecal samples, which from patients before surgery, 7 days after antibacterial drug use and 7 days after antibacterial drug withdrawal, also will be collected. All the fecal samples will be used for analyzing gut microbiota.

Total (bound plus unbound) plasma concentrations of S-warfarin and R-warfarin and vitamin K1concentrations will be measured by liquid chromatography mass spectrometry (LC-MS/MS). Metabolomics will be measured by AB SCIEX Triple TOF 5600. All these detections will be conducted in Department of Clinical Pharmacology, the First Affiliated Hospital of Soochow University, Suzhou, China. The analysis of gut microbiota will be detected by Sangon Biotech (Shanghai) Co., Ltd or Majorbio (Shanghai) Co., Ltd. This study will investigate the individualized warfarin from pharmacogenomics, pharmacokinetics and pharmacodynamics, gut microbiota and metabolomics.

The data will be analyzed by machine learning or neural networks to analyze the data.

Expected Results and Conclusions *(optional)*

Expected the polymorphisms of pharmacogenomics (CYP 2C9, VKORC1, and CYP4F2 etc.), vitamin K1concentrations, and other biomarkers from gut microbiota and metabolomics were confirmed as the effect factor for the individual variability of warfarin. And an artificial intelligence model of warfarin would be established.

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