Warfarin genetic biomarkers in VKORC1 and CYP2C9*2 genes: advancing personalized anticoagulant therapy using electrochemical genosensors

Tiago Moreira [1, 2], Eduarda Pereira [1, 2], Inês F. Costa [1, 2], António J.S.F. Sousa [1, 2], Stephanie L. Morais [2], Hygor Ferreira-Fernandes [3], Giovanny R. Pinto [4], Marlene Santos [1], M. Fátima Barroso [2*]

[1]*CISA, ESS, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal*
[2]*REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 4249-015, Porto, Portugal*
[3]*Instituto de Educação, Ciência e Técnologia do Piauí (IFPI), Departamento de Informação, Ambiente, Saúde e Produção Alimentícia, Teresina, PI, Brazil*
[4]*Grupo de Estudos em Genética Humana e Médica (GEHMED), Laboratório de Genética e Biologia Molecular, Departamento de Biomedicina, Universidade Federal do Delta do Parnaíba (UFDPar), Parnaíba, PI, Brazil*
mfb@isep.ipp.pt

Background: The genetic variants of vitamin K epoxide reductase complex (VKORC1) and in the cytochrome CYP2C9*2 genes have been identified to influence the anticoagulant warfarin and influence its plasmatic levels. Therefore, the pharmacogenetic information on these genes is useful for reducing warfarin adverse reaction [1]. **Objectives**: This work addresses the development of disposable electrochemical genosensors able of detecting single nucleotide polymorphism (SNP) in the VKORC1 and CYP2C9*2 genes. **Methodology**: The genosensor methodology implied the immobilization of a mixed self-assembled monolayer (SAM) linear DNA-capture probe and mercaptohexanol (MCH) onto screen-printed gold electrodes (SPGE). To improve the genosensor's selectivity and avoid strong secondary structures, that could hinder the hybridization efficiency, a sandwich format of the DNA allele was designed using a complementary fluorescein isothiocyanate-labelled signaling DNA probe and enzymatic amplification of the electrochemical signal [2]. **Results**: The developed electrochemical genosensors were able to discriminate between the two synthetic target DNA targets in both SNPs, as well as the targeted denatured genomic DNA. Several analytical parameters, such as DNA capture probe, 6-mercaptohexanol (as spacer) and antibody concentrations, as well as hybridization temperature and incubation time, were optimized. Using the best analytical conditions calibration curves employing increasing DNA target concentractions were ploted. Polymerase Chain Reaction (PCR), will be used for further validation of the electrochemical genosensor. **Conclusions**: Disposable electrochemical genosensors capable of detecting and distinguishing between two synthetic CYP2C9*2 and VKORC1 polymorphic sequences, with high selectivity and sensibility and in various concentrations, was developed. The functionality of these analytical approaches as alternative to the conventional genotyping methodologies can relieve the public health-care systems and, hopefully, prevent ADRs related to CDV episodes.

Keywords: VKORC1 gene; CYP2C9*2 gene; warfarin; electrochemical genosensors; molecular biology;

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