Development of electrochemical genosensors for the CYPC*2 gene polymorphism detection

Sousa, António J.S.F., CISA|ESS, Centro de Investigação em Saúde e Ambiente, Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 400, 4200-072, Porto, Portugal
Costa, Inês F., CISA|ESS, Centro de Investigação em Saúde e Ambiente, Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 400, 4200-072, Porto, Portugal
Banegas, Rodrigo S., Instituto Federal Catarinense (IFC) – Campus Camboriú, Rua Joaquim Garcia s/n. Camboriú, Santa Catarina, Brazil
Morais, Stephanie L., REQUIMTE
Magalhães, Júlia, REQUIMTE
Domingues, Valentina, REQUIMTE
Delerue-Matos, Cristina, REQUIMTE
Ramos-Jesus, Joilson, Grupo de Estudos em Genética Humana e Médica (GEHMED), Laboratório de Genética e Biologia Molecular, Departamento de Biomedicina, Universidade do Delta do Parnaíba (UFDPar), Parnaíba, PI, Brazil
Ferreira-Fernandes, Hygor, Grupo de Estudos em Genética Humana e Médica (GEHMED), Laboratório de Genética e Biologia Molecular, Departamento de Biomedicina, Universidade do Delta do Parnaíba (UFDPar), Parnaíba, PI, Brazil
Pinto, Giovanny R., Grupo de Estudos em Genética Humana e Médica (GEHMED), Laboratório de Genética e Biologia Molecular, Departamento de Biomedicina, Universidade do Delta do Parnaíba (UFDPar), Parnaíba, PI, Brazil
Santos, Marlene, CISA|ESS, Centro de Investigação em Saúde e Ambiente, Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 400, 4200-072, Porto, Portugal
Barroso, M. Fátima, REQUIMTE

Abstract

Pharmacogenetic studies search for heritable genetic polymorphisms that influence responses to drug therapy. Pharmacogenetics has many possible applications in cardiovascular pharmacotherapy including screening for polymorphisms to choose agents with the greatest potential for efficacy and least risk of toxicity. Pharmacogenetics also informs dose adaptations for specific drugs in patients with aberrant metabolism [1].

Cardiovascular diseases (CVD) are considered one of the leading causes of death worldwide. To prevent cardiovascular complications and further loss of life oral anticoagulants (e.g., warfarin) are frequently prescribed to patients. Nevertheless, warfarin therapeutic agent presents narrow therapeutic windows with well-documented health risks. Some of these dose-responses are a result of specific single-nucleotide polymorphism (SNP) genetic variations present in a patient´s DNA. Among them, determined SNP in the cytochrome P4502C9 (CYP2C9), namely the CYP2C9*2, gene has been identified as dose-response altering SNP. Therefore, the need for a rapid, selective, low-cost and in real time detection device is crucial before prescribing any anticoagulant.

In this work an analytical approach based on electrochemical genosensor technique is under development to create a low-cost genotyping platform able to genotype SNPs related with the therapeutic response of warfarin. Analyzing public databases, two specific 71 bp DNA probes, one with adenine (TA) and other with guanine (TG) SNP genetic variation were selected and designed. The design of this electrochemical genosensor consists of ssDNA immobilization onto gold surfaces that act as the SNPs complementary probes. The hybridization reaction is performed in

a sandwich format of the complementary ssDNA, using an enzymatic scheme to amplify the electrochemical signal. The electrochemical signal was performed by using chronoamperometric technique.