INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO
MESTRADO EM BIORRECURSOS




Polimorfismos genéticos de interesse na cardiologia: uma visão geral na Europa

TIAGO FILIPE COELHO GONÇALVES
novembro de 2020

P.PORTO

Genetic polymorphisms of interest in cardiology: an European overview

Tiago Filipe Coelho Gonçalves

Dissertação Submetida como requisito parcial para a obtenção do grau de Mestre em Biorrecursos

November 2020

Supervisor:

Dra. Valentina Domingues

Dra. Fátima Barroso

Dr. Giovanny Pinto

AKNOWLEDGEMENTS

I would first like to thank my supervisors Dr. Valentina Domingues, Dr. Fátima Barroso, and Dr. Giovanny Pinto for their relentless willingness to help me and to bring this study to a successful conclusion. At any time, they were willing to help me or answer any questions I had and without them I could not, under any circumstances, defend my master's thesis.

Also, I would like to thank Stephanie Morais for giving me precious help on the laboratory procedures and to discuss with me any aspect of this study.

For my family and girlfriend Sara Vara would also like to leave a word of thanks for their support and patience during times of greatest discouragement and sadness. Undoubtedly, your emotional support was essential to keep my spirits up.

Last but not least, I want to thank my friends who have done this walk with me over these 5 years. I am referring to Marco Martins, Paulo Joel Nunes, Filipe Fernandes, João Moreira and Miguel Tavares who gave me a lot of laughs, deep conversations that will make me miss them immensely. We grew up together and today we can be proud of everything we have achieved.

This work was funded by the Bilateral Cooperation FCT/CAPES 2018/2019 Processo 4.4.1.00 CAPES (CAPES-FCT2017484310P 200.137.174.210).

PREAMBLE

The initial aim of this project was to investigate the influence of certain genetic polymorphisms on heart disease in the Portuguese population. Specifically, this study intended to study the frequency of *VKORC1* and *CYP2C9* genes' polymorphisms and their impact on an anticoagulant drug's metabolization used to treat and prevent cardiovascular diseases. The drug in study was the warfarin, an antagonist of vitamin K widely used to treat and prevent cardiovascular diseases through the inhibition of vitamin K production. The vitamin K inhibition will consequently decrease clotting factors II, VII, IX, and X presence on the coagulation cascade, preventing clot formation or expansion [1]–[3].

The reason to perform this work was the urgent need for new technologies to rapidly predict a customized drug dose for each patient. A warfarin dose customization allows the minimization of any adverse effect through the prescription of the lowest drug dose to prevent clot formation. The main issue found related to warfarin is its narrow therapeutic index that makes it impossible to administer a standardized dose to a patient, which means that a specific dose for an individual might not apply for another. The variation in the warfarin dose requirement will depend on the patient's vitamin K intake, age, and the presence of the *VKORC1* and *CYP2C9* genes' polymorphisms. [4].

This research would occur by the optimization of the DNA samples' extraction and purification, followed by the optimization of PCR amplification parameters. Moreover, the intention was to perform DNA electrophoresis run with agarose gel and then detect the studied polymorphisms on the amplified DNA using electrochemical genosensors.

However, the suspension of this experimental work was mandatory due to the COVID-19 pandemic. The president of Instituto Politécnico do Porto and the other responsible directed the suspension of all the laboratory and classroom activities to assure the safety of students, teachers, and employees. Even if this compromises the completion of this project, there are more relevant issues and values to consider when fighting a pandemic.

As laboratory activities were not feasible, there was the need to find a project as challenging as this. The plausible solution found was to change the focus of this project to compile in an article review the frequency of the *CYP2C9*, *VKORC1*, and *CYP4F2* genes polymorphisms in every European country.

SUMÁRIO

As doenças cardiovasculares são a principal causa de morte na Europa, representando cerca de 3.90 milhões de mortes anuais. O anticoagulante mais prescrito para tratar e prevenir complicações advindas destas doenças é a varfarina. Por outro lado, a varfarina é também o fármaco mais associado a efeitos nefastos devido à prescrição de dosagem incorreta. A variação da dose de varfarina é explicada em 56% pela presença dos polimorfismos nos genes *VKORC1, CYP2C9* e *CYP4F2*. Esta significativa variação na dose de varfarina realça a importância da farmacogenética na individualização da dose prescrita. Este estudo foi realizado com o intuito de compilar informação acerca da presença destes polimorfismos em países europeus e avaliar o seu impacto na variação da dose de varfarina necessária.

Estes dados foram obtidos através de uma pesquisa sistemática nas bases de dados PUBMED e Web of Knowledge através do uso das seguintes palavras-chave: [*País da Europa*, *gene* e polymorphisms] e [*Nacionalidade de cada país da Europa*, *gene* e polymorphisms]. Esta pesquisa foi levada a cabo de julho de 2020 a agosto de 2020, da qual foram obtidos 119 artigos válidos. Os critérios de exclusão foram artigos de revisão sistemática, meta-análises e estudos que não estivessem escritos em inglês. Para além destes, estudos genotípicos conduzidos em pacientes e em animais foram também descartados.

No que diz respeito ao gene VKORC1 avaliou-se a frequência dos polimorfismos -1639 G>A, 1173 C>T, e 3730G>A. Relativamente ao gene CYP2C9 avaliou-se a frequência dos polimorfismos CYP2C9*2 e CYP2C9*3. Por último, o polimorfismo V433M foi o único do gene *CYP4F2* avaliado nas populações europeias.

Para simplificar a análise dos polimorfismos genéticos nos países europeus, estes foram agrupados em seis subdivisões geográficas: Norte, Este, Sudeste, Centro, Sul e Oeste.

As populações estudadas de países do sudeste europeu apresentaram uma maior frequência para os polimorfismos -1639 G>A (de 39.9% a 53.4%) e 1173 C>T (de 46.7% a 50.0%) do gene VKORC1. No sentido inverso, as populações estudadas do sul, centro e oeste europeu apresentaram uma frequência para os polimorfismos -1639 G>A, e 1173 C>T do gene VKORC1 menor do que 43.1% e 44.8%, respectivamente. As populações do sudeste europeu mostraram uma alta frequência para os polimorfismos *CYP2C9*2 (de 12.5% a 18.6%), e *CYP2C9*3 (de 7.3% a 10.9%). Além disso, as populações estudadas do norte e este europeu apresentaram uma baixa frequência para os polimorfismos *CYP2C9*2 (de 8.8% a 14.3%), and *CYP2C9*3 (de 4.6% a 7.4%).

De acordo com a frequência dos polimorfismos -1639 G>A e 1173 C>T do gene *VKORC1*, um indivíduo de um país do sudeste europeu tem maior probabilidade de necessitar de uma menor dose de varfarina. Da mesma forma que, atendendo às frequências dos polimorfismos *CYP2C9*2 e *CYP2C9*3, um indivíduo do sul da Europa tem maior probabilidade de requerer uma dose mais baixa de varfarina.

Em relação aos polimorfismos V433M (CYP4F2) e 3730 G>A (VKORC1) não é possível retirar qualquer conclusão acerca da sua frequência em países europeus, devido à escassez de informação.

PALAVRAS-CHAVE: *VKORC1*, *CYP2C9*, *CYP4F2*, genes, polimorfismos, farmacogenética e Europa.

ABSTRACT

Cardiovascular diseases (CVDs) are the leading cause of mortality in Europe accounting for 3.90 million deaths each year. The most widely used anticoagulant to treat and prevent CVDs events is warfarin. However, warfarin is also the drug most associated with adverse reactions due to incorrect dosage. This warfarin dose variation is explained by 56% in the presence of the Vitamin K epoxide reductase complex subunit 1 (*VKORC1*), Cytochrome P450 2C9 (*CYP2C9*), and Cytochrome P450 4F2 (CYP4F2) genes polymorphisms. Such a narrow therapeutic index for warfarin justifies why pharmacogenetics are indispensable for dose customizing. Therefore, this study aimed to compile the available data regarding these polymorphisms' frequency in European countries and to assess their impact on the warfarin requirement dose.

This data was obtained through a systematic search carried out in PUBMED, and Web of Knowledge using the following keywords: [*European country*, *gene* and polymorphisms] and [*each European country nationality*, *gene* and polymorphisms]. This search was conducted between July 2020 and August 2020, from which were retrieved 119 valid papers. The exclusion criteria were systematic reviews, meta-analysis, and non-writing English papers, as well as genotypic studies conducted in patients, and animals.

Concerning the *VKORC1* gene, the obtained studies evaluated the frequency of the -1639 G>A, 1173 C>T, 3730G>A polymorphisms in European populations. Regarding the *CYP2C9* gene, the frequency of the *CYP2C9**2 and *CYP2C9**3 polymorphisms was assessed. Finally, the *CYP4F2* gene only had the frequency of the V433M polymorphism assessed in European populations.

To simplify the analysis of polymorphisms' frequency, the European countries were grouped into six geographical subdivisions (Northern, Eastern, Southeastern, Central, Southern, and Western).

The studied populations from Southeastern Europe countries showed a higher frequency either for the VKORC1 gene -1639 G>A (39.9% to 53.4%) and 1173 C>T (46.7% to 50.0%) polymorphisms. Conversely, the Southern, Central, and Western European studied populations presented a frequency for the VKORC1 gene -1639 G>A, and 1173 C>T polymorphisms lower than 43.1%, and 44.8%, respectively. The studied populations from Southern Europe displayed a higher frequency for the *CYP2C9**2 (12.5% to 18.6%), and *CYP2C9**3 (7.3% to 10.9%) polymorphisms. Also, the Northern and Eastern European populations presented a lower frequency for the *CYP2C9**2 (8.8% to 14.3%), and *CYP2C9**3 (4.6% to 7.4%) polymorphisms.

Concerning the frequency of the *VKORC1* gene -1639 G>A and 1173 C>T polymorphisms, one individual from Southeastern Europe is more likely to require a lower warfarin dose. Simultaneously, the frequencies for the *CYP2C9**2, and *CYP2C9**3 polymorphisms suggest that one individual from Southern Europe is more prone to require a lower warfarin dose.

It is not possible to draw any conclusions regarding the frequency of V433M (*CYP4F2*), and 3730 G>A (*VKORC1*) polymorphisms due to due to the scarcity of studies assessing their frequency in European countries.

KEYWORDS: *VKORC1*, *CYP2C9*, *CYP4F2*, genes, polymorphisms, pharmacogenetics, and Europe.

Index

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CHD – Coronary Heart Disease

CT – Control Time

CVDs – Cardiovascular Diseases

CYP1A1 - Cytochrome P450 1A1

CYP1A2 - Cytochrome P450 1A2

CYP2C19 - Cytochrome P450 2C19

CYP2C9 - Cytochrome P450 2C9

CYP3A4 - Cytochrome P450 3A4

CYP4F2 - Cytochrome P450 4F2

EU – European Union

GDP – Gross Domestic Product

GGCX - Gamma-glutamyl Carboxylase

INR - International Normalized Ratio

ISH - International Society of Hypertension

ISI – International Sensitivity Index

LDL - Low-Density Lipoprotein.

MAF – Minor Allele Frequency

PT – Prothrombin Time

SES – Socioeconomic Status

SNPs - Single-nucleotide polymorphisms

UK – United Kingdom

USA – United States of America

VK – Vitamin K

VK1 – Phylloquinone

VK2 – Menaquinones

VKORC1 – Vitamin K Epoxide Reductase

WHO – World Health Organization

1. INTRODUCTION

1.1. Cardiovascular diseases in Europe

Cardiovascular diseases (CVDs) are the leading cause of mortality and disability worldwide whether in developed, low or middle-income countries. Even though they affect women in a higher proportion than men. Every year, about 32.4 million CVDs events occur, being the myocardial infarctions and strokes the most reported cases [5], [6].

In Europe, according to data from 2017, CVDs estimate for 3.90 million deaths each year, representing 45% of the total. The most prevalent CVDs are coronary heart disease (CHD) and stroke. CHD is the leading cause of death (19% of all deaths) among the European countries accounting for about 1.74 million deaths a year. On the other hand, stroke ranks second on the list of the most lethal causes of death, representing almost 1 million deaths every year [7].

CVDs are also the leading cause of premature death across Europe. Annually, about 1.3 million people under the age of 75 years die from complications related to CVDs (35% of total deaths). Concerning people under 65 years old, CVDs are the second cause of death accounting for 192 thousand deaths (22% of total) [7].

The CVDs have their origin in a phenomenon named atherosclerosis. This event consists of an artery's inside narrowing due to the plaque build-up that develops arterial occlusion over time (Figure 1.1). The most severe cases of atherosclerosis manifest their symptoms when the consequences for the patients are irreversible. It mainly results in unpredictable and sometimes fatal acute coronary events (heart attacks) and cerebrovascular events (strokes). [5], [6].

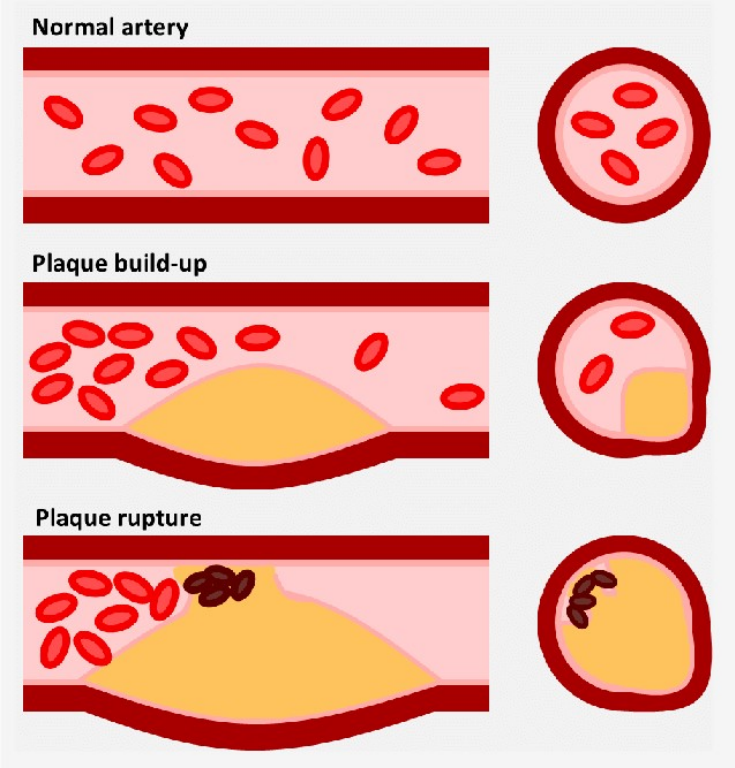

Figure 1.1 - Illustrated representation of plaque build-up, which over the years will lead to arterial occlusion [8].

The sudden occurrence of the clinical events mentioned is more likely to happen whether the individual adopts risky behaviours (such as smoking, sedentary lifestyle, lack of balanced nutrition, excessive alcohol consumption, and stress). These risk factors lead to higher blood pressure and low-density lipoprotein (LDL) cholesterol. These factors combined with an eventual family's historical predisposition to CVDs, will increase the probability of a CVD event to occur. Therefore, a timely change of risk habits would reduce clinical events and premature deaths by a third in flagged people with CVDs, and in people at high CVD risk due to one or more factors [5], [6], [9].

The World Health Organization (WHO) and the International Society of Hypertension (ISH) developed prediction charts to assess the incidence of CHD, stroke, and peripheral events. These charts apply for first and recurrent cases to prevent and control CVDs in low– and middle– income countries. These charts enable equity between low– and middle– income countries with high-income countries as they balance their populations' level of CVDs risk assessment. [10].

The risk prediction charts calculate the combined risk of developing a CVD based on the individual's risk factors (age, gender, smoking habits, blood pressure, blood cholesterol, blood sugar). As seen in Figure 1.2, the risk prediction charts enable an individual to be classified according to their risk of suffering a CVD event in the following ten years. The darker the assigned colour, the greater the risk of an individual to suffer a clinical event. For those individuals, whose score results in high cardiovascular risk, the guidelines recommend the administration of vitamin K (VK) antagonists and an abrupt change on the risk behaviours. Also, it is crucial to have in mind that people with previous CVDs occurrences have a much higher probability of suffering from other clinical events. On the other hand, if the risk is low, there is no need for such drastic changes, but it is recommended that the individual adjusts some behaviours, such as smoking [11].

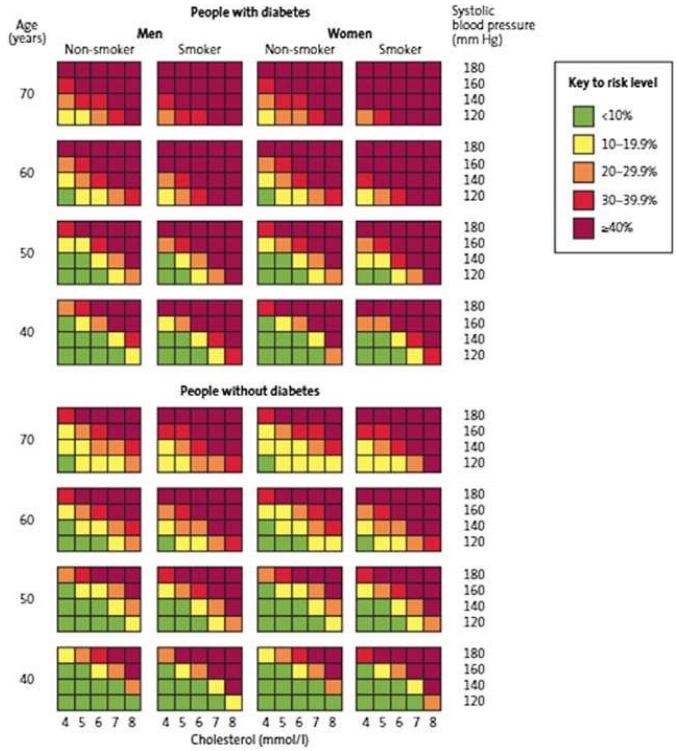

Figure 1.2 – WHO and ISH cardiovascular risk prediction charts [12].

The individuals' socioeconomic status (SES) and their country's income will significantly impact their predisposition to suffer from CVD. Men and women living in high-income countries with low SES are more susceptible to CVDs than people with high SES. The principal reasons for this to happen seems to be the psychosocial factors, the influence of area of residence, and the health inequalities and inequities in the referred countries. The same is replicated in low-income and middle-income at a grander scale not only because of the higher revenue disparities, but also because social aid and health services are often not guaranteed [13].

The solution to this health problem is noticeable, but its implementation is arduous. As middle-income countries become wealth, CVDs patterns are improved, and with that, their populations' SES reaches higher levels. It is possible to replicate in lower-income countries what has been done in higher-income countries. This can be achieved by incorporating SES into screening and risk calculation to reduce psychosocial and behavioural risk, enabling equal care for everyone [13], [14].

There is a variety of factors that will influence the variability in disease frequency. It may depend on the countries' progress rhythm and where that progress leads. For example, when countries develop from agrarian to an industrial situation, there are changes at an environmental, social, and structural scale. This will drive to the increase of people's lifespan but, on the other hand, it will lead a higher exposure to risk factors for chronic diseases. The determining part of this subject is to find the balance between developing and the increase of risk factor levels to define the incidence frequency and mortality caused by CVD [14].

Simultaneously, other circumstances may influence the rising of mortality caused by CVDs in some countries. Some nations face additional pressures like war or infectious diseases while individuals from other countries may have or not a genetic predisposition for CVD risk (e.g., in Japan, despite the CVDs' risk levels increment over the last years, its incidence is still 3-fold lower than in the United States of America (USA)) [14].

Among the European countries, particularly the European Union (EU) countries (which includes the United Kingdom in the 2016 year), Iceland, Norway, Switzerland, Serbia, and Turkey there are significant income differences. This income difference could correlate to different CVDs' mortality rates, even if some of these countries are part of an exclusive economic zone and all are geographically located on the same continent [15].

There are a few ways of measuring a country's economic activity, living and development standards. However, the most consensual and used indicator is the Real Gross Domestic Product (GDP) per capita, even though it does not consider the adverse effects of economic activity and the unpaid household. The distinction between Real GDP and GDP is that Real GDP takes inflation into account. Therefore, GDP is an indicator that measures the final output of all goods and services that a country's economy produces over a year divided by its population's number [15]. In the year 2016, the EU countries CVDs' mortality rate represented 37.1% of the total number, equivalenting to 1.68 million deaths. However, the CVD's mortality rate disparity amongst the EU countries can be as broad as 23.9% in Denmark to 66.2% in Bulgaria. Part of this referred disparity could find an explanation on the Real GDP per capita in each country [15], [16].

Looking at Figure 1.3 is possible to observe that in countries whose Real GPD per capita is lower than 2000€, the CVDs mortality rate is, generally, as higher as lower the Real GDP is. This correlation is specifically noted on Balkan, Baltic, and Eastern European (Poland, Czech Republic, Hungary) countries whose income is low, while presenting the highest CVDs' mortality rate. This group of countries show a CVDs' mortality rate above 43.3% and an income per capita below 17000€ [15], [16].

Still, countries with higher income per capita may show CVDs' mortality rates similar to lower income countries due to social, cultural, eating behaviours and health factors. There are good examples of countries with higher CVDs' mortality rates despite showing higher value for Real GDP per capita as is the case of Austria and Nordic countries. Austria shows a CVDs' mortality rate comparable to all the studied Eastern countries despite the Austria's income (36430€) being 49.1% to 70.1% higher. Concerning the Nordic countries, although presenting a high value for Real GPD per capita, only Denmark stands out for showing the lowest CVDs' mortality rate (23.9%). Simultaneously, Norway, Iceland, Sweden, and Finland compare their mortality rate to countries whose income is much lower. A great example of this is Portugal that despite having a medium-low income (17010€), has a similar or lower CVDs' mortality rate than some Nordic countries whose income is 51.8% to 75% higher.

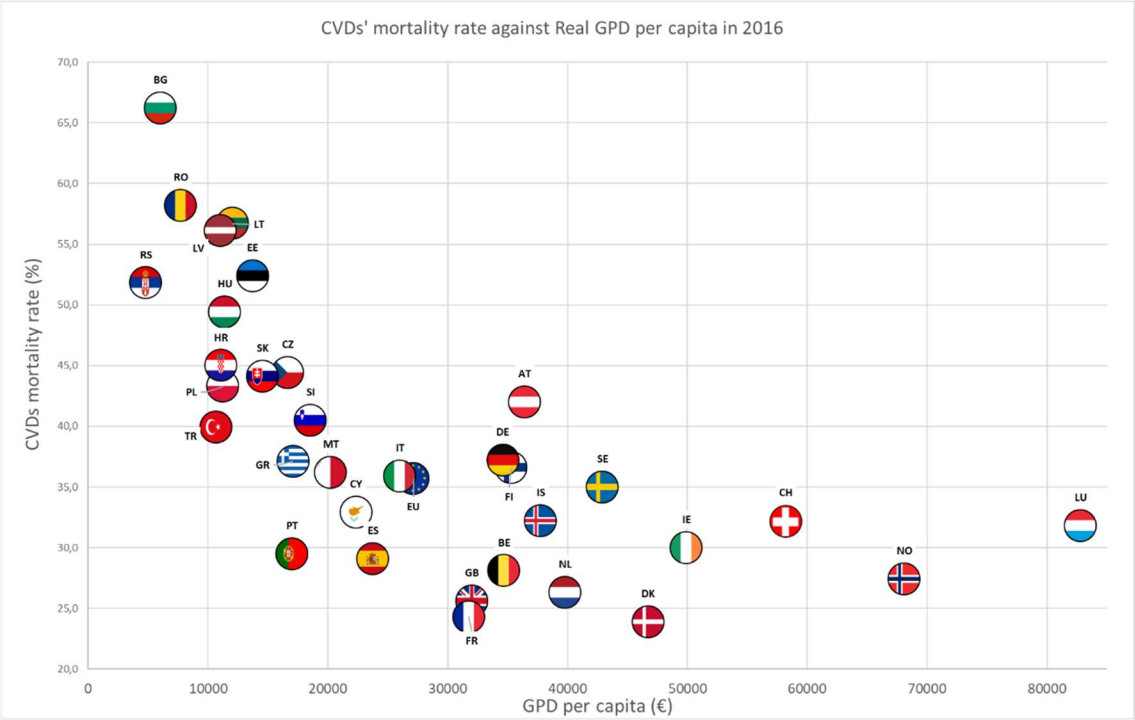

Figure 1.3 – CVDs mortality rate against Real GPD per capita in the 2016 year. Adapted data from [15]

1.2. Coagulation cascade

The coagulation cascade is a sequence of events in which chain-activated anticoagulant proteins are involved, allowing haemostasis to occur. The rapid prevention and cure of unexpected bleeding are due to the existence of two distinct pathways (extrinsic and intrinsic). These pathways converge on a common pathway, leading to the conversion of fibrinogen into fibrin. The haemostasis process occurs in two different phases. The first phase of haemostasis is the organism's first response to bleeding, forming an aggregation of platelets at the damaged site. The secondary haemostasis is the organism's reinforcement response by the intrinsic and extrinsic pathway in the damaged place. This response will combine fibrin strands, binding platelets together to stabilize the platelet plug [1], [3], [17]–[19].

The Figure 1.4 shows the intrinsic, extrinsic, and common pathways in the coagulation cascade, and how the involved proteins interact with each other. The factors of the intrinsic pathway (VII, IX, XI, and XII), the extrinsic pathway (III and VII), and the common pathway (I, II, V, and X), when not activated, flow into the bloodstream as zymogens. Also, some of them (factors II, VII, IX, X, XI, and XII) will be then activated into serine proteases to catalyse the conversion of the remaining zymogens into serine proteases [1], [3], [17]–[20]

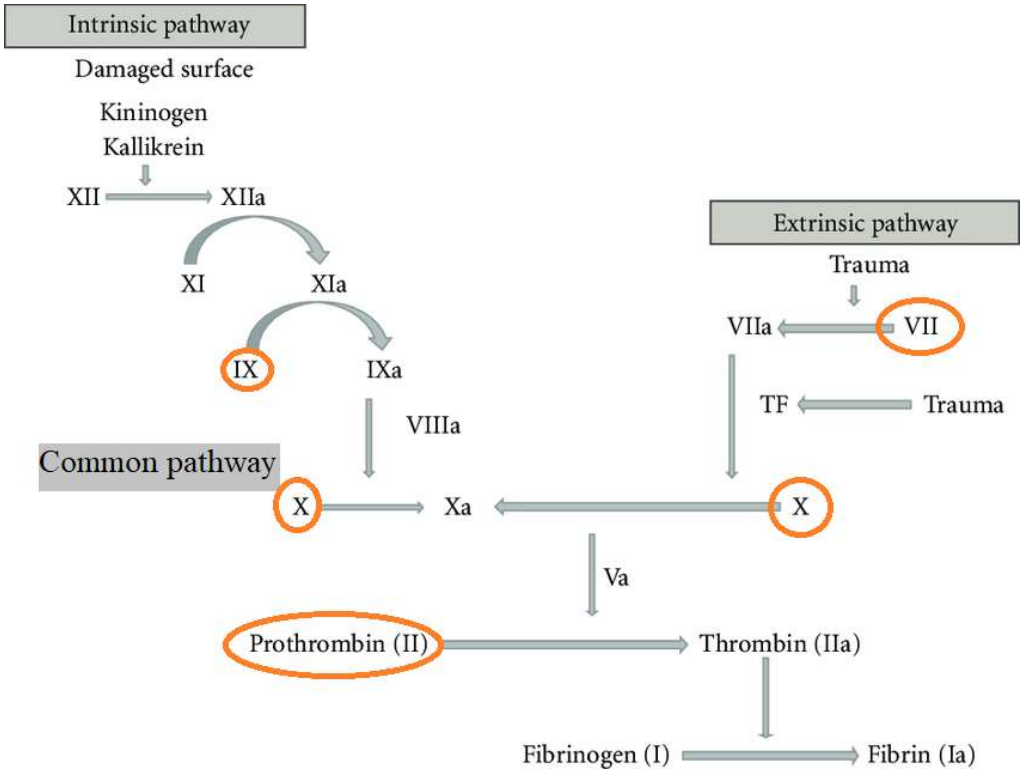

Figure 1.4 - Illustrated representation of coagulation cascade. The clot factors surrounded with orange colour are the factors inhibited by warfarin Adapted from [21]

The intrinsic pathway involves the largest number of clotting factors compared to the extrinsic. The exposure to endothelial collagen, which only happens when there is endothelial damage, unleashes the activation of the serine protease Factor XIIa. The Factor XIIa will then catalyze the activation of Factor XI into Factor XIa, which in turn will activate Factor IXa. Factor IXa will next stimulate the Factor X activation into Factor Xa, where the intrinsic pathway and the extrinsic pathway fuse [20].

On the other hand, the extrinsic pathway is the shorter pathway. This process begins with the damage of vessels that will make the endothelial cells to release the tissue factor (Factor III), allowing the activation of Factor VII zymogen into Factor VIIa. Factor VIIa will, in its turn, activate factor Xa, and at this point, the intrinsic and extrinsic pathways come together [20].

As previously mentioned, the common pathway results from the meeting of the intrinsic and extrinsic pathways. In this way, the common pathway starts at the activation of factor X to factor Xa through cleavage by the tenase. Tenase can either be a complex of the extrinsic pathway, which includes the tissue factor, Factor VIIa, and Ca^{2+}, or intrinsic pathway, containing Factor IXa, CoFactor VIII, phospholipids, and Ca^{2+}. Therefore, Factor II (prothrombin) is then cleaved into its active form Factor IIa (thrombin) by Factor Xa in conjunction with the CoFactor V. Factor IIa will then catalyze Factor I (fibrinogen) activation into Factor Ia (fibrin). The fibrin subunits will, in turn, come together to form the fibrin strands. The fibrin strands with the action of Factor XIII, will create a fibrin mesh to stabilize the platelet plug [1], [3], [17]–[19], [22].

1.3. Warfarin and oral anticoagulants

Warfarin is, nowadays, the most prescribed oral anticoagulant in the world to treat thromboembolic disorders, but it was primarily used as a rodenticide in 1952. Two years later, warfarin started being marketed to prevent and control thromboembolism prophylaxis and venous thromboembolism. The intake of this drug is one of the most common causes of emergency for adverse drug reactions. This happens due to its narrow therapeutic index, multifactorial etiology exposure, and high usage, making dose standardization impossible. Briefly, this means that a specific dose for an individual might cause bleeding events, while the same dose might cause blood clot in other. [1]–[3].

As warfarin (Figure 1.5) pharmacokinetics is not understood yet, there is the need to know the initial response to therapy. This drug is a racemic mixture (a substance that contains equal amount of left- and right-handed enantiomers of a chiral molecule) of -S and -R stereoisomers that will bound 99 % to albumin. S-warfarin is transformed by Cytochrome P450 2C9 (CYP2C9 into inactive metabolites while R-warfarin by Cytochrome P450 1A1 (CYP1A1), Cytochrome P450 1A2 (CYP1A2), Cytochrome P450 2C19 (CYP2C19), and Cytochrome P450 3A4 (CYP3A4). These enantiomers will act as VK epoxidase reductase complex inhibitors, being the S-warfarin 3 to 5-fold more efficient than the R-warfarin enantiomer [1]–[3], [23]–[25].

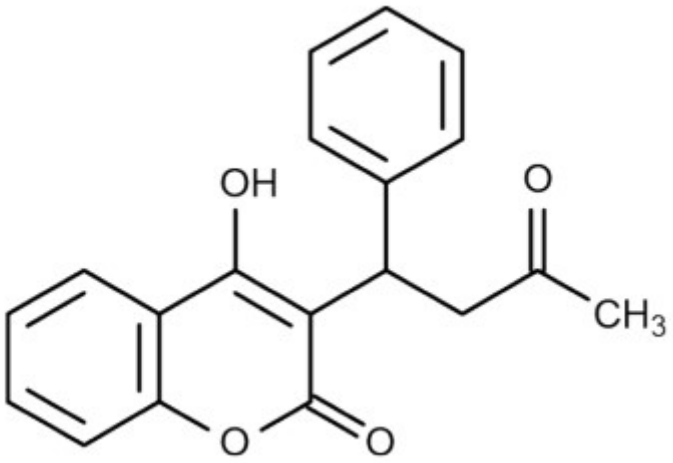

Figure 1.5- Chemical structure for warfarin

The anticoagulation treatment aims to administer the drug's lowest possible dose to prevent clot formation or expansion. Warfarin acts as an antagonist of VK, which is a fundamental element on the synthesis of clotting factors II, VII, IX and X, well as anticoagulant proteins C, S and Z. The anticoagulation state occurs due to the inactivation of the carboxylation of some glutamic acid residues by VK antagonists. This carboxylation process requires a reduced form of VK to allow the production of clotting factors and anticoagulant proteins. Therefore, in the presence of VK antagonists, the rate of production of clotting factors and proteins will reduce by 30% to 50%, allowing anticoagulation [1]–[3].

Warfarin has a fast and almost complete absorption through the gastrointestinal tract, whose effect lasts from 2 to 5 days. After the first intake, warfarin will bond to plasmatic albumin, where its maximum presence will be detected within 1 hour. However, its highest antithrombotic effect will only manifest about 48 hours later with an average half-life of 40 hours. The liver and the kidneys carry out this drug metabolization and, after being transformed into inactive metabolites, will be excreted through urine and faeces [1]–[3], [23]–[25].

There is a known but not quite understood interindividual variation response on the warfarin administration due to the genetic, clinical, physical, SES and environmental factors. The discrepancy in patients' warfarin dose can be as broad as 0.6 mg/day to 15.5 mg/day. Individuals on warfarin therapy usually are from 45% to 63% of the time within the therapeutic range (upper limit drug concentration above which adverse effects are likely, and by a lower limit below which warfarin is most often not effective). Yet, the proportion of patients showing a positive response to warfarin administration in the treatment of CVDs is significantly high, being from 50% to 75%. On the other hand, the percentage of patients who suffered from adverse reactions is 15% [1], [3], [26], [27].

VK is a fat-soluble vitamin with a methylated naphthoquinone nucleus (menadione) and a variable aliphatic side chain at the 3′ position that can assume two natural forms, namely Phylloquinone (VK1) and Menaquinones (VK2). VK in its reduced form plays an indispensable role in blood clotting since it is in the VK cycle that clotting factors II, VII, IX and X used in the coagulation cascade are produced. [28].

The VK cycle (Figure 1.6) begins by the reduction of dietary VK form. This reduction occurs through the action of quinone reductase that will catalyse the donation of two electrons by NADPH, giving rise to VK hydroquinone. Afterward, VK hydroquinone will be the cofactor alongside carbon dioxide and oxygen for the carboxylation step by giving its electrons to gamma-glutamyl carboxylase (GGCX). The electron acceptance by GGCX will allow the conversion of the coagulation factors and anticoagulation proteins onto their functional forms. This occurs because of the addition of a carboxyl group onto the end of their glutamic acid residues [3], [18], [29], [30] .

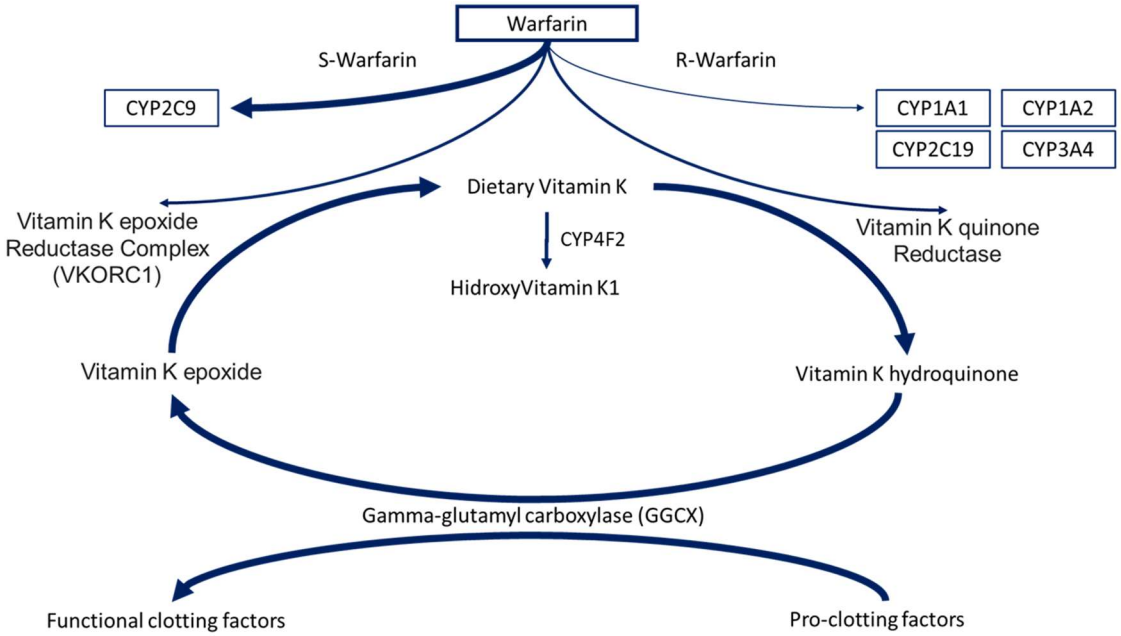

Figure 1.6 - VK cycle and respective warfarin effect on this process. Adapted from [25], [29].

VK is, after the carboxylation step, in an oxidized form to which is given the name of VK epoxide. From this moment on, vitamin K epoxide reductase (VKORC1) will stimulate the conversion of the VK epoxide to the original dietary VK form, through the donation of electrons from a thiol group. Also, the Cytochrome P450 4F2 (CYP4F2) enzyme is known for regulating the excess of VK1 through its oxidation into hidroxyVK1, enabling the removal of active VK1 from the VK cycle [3], [18], [31], [32]. A single VK molecule can be part of innumerable VK cycles, and warfarin acts on the coagulation process by restraining its regeneration. As can be seen in Figure 1.6, warfarin inhibits the function of the VKORC1 and quinone reductase enzymes by binding to their active site in a still uncertain way. Therefore, the coagulation factors' activation on the coagulation cascade will be inhibited [1], [3], [18], [19], [33].

As noted, warfarin affects the VK cycle by inhibiting the activity of the enzymes VKORC1 and quinone reductase. This warfarin action will inhibit the cyclic interconversion of VK, which means it will lower the production of the VK hydroquinone. In the absence of VK hydroquinone, GGCX is then unable to proceed to the carboxylation step that converts non-functional coagulation factors and anticoagulant proteins into functional ones. This happens because Ca^{2+} and the phospholipid membranes will not be able to bind accordingly to proteins, which will discontinue the coagulation cascade [34], [35].

1.4. Coagulation tests

Studies prove that the warfarin's antithrombotic effect is somehow dissociated from its anticoagulant effect. Also, some studies suggest that the reduction of factors II and X are more preponderant than the rest. Warfarin, in a study carried out in rabbits, showed an antithrombotic effect after six days of treatment. On the other hand, the anticoagulant effect rises only after two days. The antithrombotic effect necessarily requires a decrease in the values of prothrombin (factor II). Factor II presents a half-life of 60 to 72 hours, which is much lower than that presented by the other factors with 6 to 24 hours of half-life [35], [36].

The adverse reaction of administering inappropriate warfarin doses is haemorrhage. Haemorrhage is directly linked to the level of the International Normalized Ratio (INR) whose the higher value, the higher the risk of bleeding. These undesirable events caused by warfarin increase their likelihood to happen when the patient is old, has associated pathologies and has risky behaviours. In the most severe cases, the administration of VK supplements are prescribed to lessen the adverse effects of warfarin [37].

Prothrombin time (PT) assesses warfarin dose efficacy on an individual by measuring the function of the extrinsic and common pathway. The PT evaluates the reduction that warfarin causes on Factor II, VII, and X at a proportional rate to their half-life. This means that in the first days, Factor VII will suffer a higher depletion for having a lower half-life. This test is performed by triggering the fibrin clot through the addition of isolated blood plasma and Ca^{2+} with thromboplastins. The thromboplastin responsiveness will then vary according to the reduction of coagulation factors. The time that the study sample takes to clot is the PT, which then is compared to a reference clotting time of a healthy person called Control Time (CT). The comparison between PT and CT will determine if the warfarin dose is suitable to an individual [3], [26], [35], [38].

The PT testing is not standardized because several companies are producing different testing kits whose clot rates differ, making the PT impossible to compare. The standardization of this PT value is then possible due to INR (Equation 1.1). The INR is the relation between PT and CT raised to the power of the International Sensitivity Index (ISI). The ISI is the value that each manufacturer assigns to indicate a tissue factor compared to an international reference tissue factor [3], [26], [35], [38].

$$INR = \left(\frac{PT\ test}{CT}\right)^{ISI}$$

Equation 1.1 - Equation for INR

This measurement method has been widely used since the WHO recommends its application. The use of INR allows the patients' monitoring of warfarin therapy. On a subject that is not under any anticoagulant therapy, the common INR values are from 0.8 to 1.2. On the other hand, people taking VK antagonists usually show an INR range from 2.0 to 3.0 in their blood. Theferore, a higher INR value indicates a high risk of bleeding, and a lower INR value means a high risk of thrombosis. This system is extremely convenient to predict bleeding events and sudden deaths in patients suffering from atrial fibrillation and increased stroke risk [3], [26].

1.5. DNA Polymorphisms and Pharmacogenetics

The concepts of pharmacogenetics and pharmacogenomics can be confused as synonymous, but the fact is that they are distinct sciences. Pharmacogenetics studies refer to the single-gene mutations and their respective impact on the drug response. These mutations can lead to individual differences in dose-plasma concentrations for many drugs due to changes in the metabolizing enzymes, and proteins. On the other hand, the pharmacogenomics study field relies on the entire genome surveillance. This science aims to evaluate the various determinants of drug responses, studying inter-individual variations in the DNA sequence related to drug response using genomic technologies. Pharmacogenetics and pharmacogenomics are indisputably the answers for personalized medicine issue. They make possible the prescription of drugs based on the population's polymorphisms diversity, genetic mutations, and gene expression profiles [39], [40].

However, a drug's overall effect cannot only be understood and explained by pharmacogenetics and pharmacogenomics. This is only possible if these study fields are combined with the individual's physical characteristics, environmental factors, and drugs' variable adherence and metabolization [1], [3].

The use of pharmacogenetic and pharmacogenomic strategies helped to decrease the incidence of adverse reactions of some drugs (e.g., abacavir hypersensitivity syndrome in clinical practice through prospective genotyping for *HLA-B**57:01). Nevertheless, there is no customized cardiovascular test at a clinical practice level due to logistical, financial, and health care provider barriers. These tests implementation and development worsen with the inconsistent results, the focus on new cardiovascular drugs' approval, and the lack of evidence from the patient's point of view. Besides this, most VK antagonists are non-patented, which leads them to be cheaper and prescribed by many physicians [1], [3].

The so-called polymorphisms are, by definition, a variation in the genome among the population with a minor allele frequency (MAF) higher than 1% in the whole population. Polymorphisms are responsible for a variation in the human genome of around 1%, being the remaining percentage the same in every human being. These variations occur in all traits, at any segment of coding or non-coding DNA, attributing to all human beings their different characteristics [41], [42].

Single-nucleotide polymorphisms (SNPs) are the most common polymorphisms in the human genome, accounting for 10 million SNPs identified. As the name implies, SNPs are polymorphisms whose alteration is the substitution of one single nucleotide (adenine, guanine, cytosine, or thymine) in the DNA structure for another (Figure 1.7) [43], [44].

These nucleotide substitutions may emerge either on coding or non-coding regions of the gene. Within the SNPs in coding regions they may be synonymous and non-synonymous (missense or nonsense) [43]–[47].

Non-coding substitutions occur on the intronic or untranslated regions of the gene. These variations are the main responsible for disease and complex traits due to their effect on mRNA splicing. This alteration on the splicing process enables skipping exon, retention intron, and introduction of a new splice site into an exon or intron. The result of a non-coding substitution may be the gene's expression enhancement or inhibition [43], [44].

Regarding the SNPs in coding regions, there may be a synonymous nucleotide substitution without an amino acid exchange which will not alter the protein sequence (e.g., TTC codon origins Lys but TTT codon also origins Lys). On the contrary, this nucleotide substitution may also change protein sequence (TTC codon origins Lys but TGC codon origins Thr) to which is given the name of non-synonymous SNPs [45]–[47].

Regarding synonymous SNPs, it was thought that its influence on gene expression would be insignificant. However, it is now known that it affects the translation of mRNA, the enlogation rate and decoding efficiency. Concerning the non-synonymous SNPs, a missense SNP is a variation in which the substituted nucleotide will give rise to the expression of a dysfunctional or unstable protein. On the other hand, a nonsense variation gives the protein expression a premature termination codon (TGA, TAG, TAA), from which will result a truncated and unstable protein [45]–[47].

The nonsynonymous SNPs may influence the gene expression, mRNA and DNA conformation, and translational efficiency. This has a significant impact on the individuals' susceptibility to diseases, genome evolution and drug metabolism. Despite genotype factors do not explain the whole variation on the drug requirement dose, assessing the SNPs frequency on a population is still fundamental to create models to predict drugs doses [48]–[52].

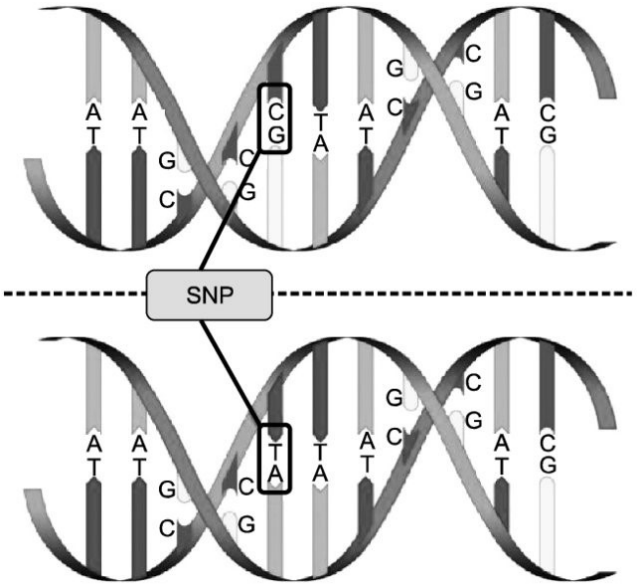

Figure 1.7- Representation of SNPs. Adapted from [53].

The SNPs are inherited from at least one of the individual's parents. All individuals receive from each parent 23 chromosomes that will be grouped in homologous chromosomes according to their genetic information. These homologous chromosomes have a locus in each allele for the same characteristic, and, depending on these alleles, it will give rise to a particular genotype [41].

When an individual carries a specific SNP in both alleles, the gene expressed is the recessive gene due to the absence of the dominant gene. On the other hand, the regular responders of a particular drug dose are the wild-type allele carriers, which means that for specific loci there is no SNP [41], [54].

The heterozygous carriers of SNPs may either carry two different SNPs in both alleles or one just one SNP in one of the alleles. The repercussion on the organism's response may change the drug requirement dose. However, when the wild-type allele is present, the impact on the drug requirement dose will not be that significant as it is when only mutant alleles are present [41], [54].

An individual with predisposition to suffer from a specific disease due to the presence of specific SNPs may never develop that pathology. This happens because the disease's onset results from the interaction between genotype, clinical, and environmental factors [48]–[52].

1.6. Polymorphism impact in Antagonist Vitamin K metabolism

The variability in warfarin dosage caused by genetic factors is not yet fully understood due to the immensity of SNPs that exist for the *VKORC1*, *CYP2C9*, and *CYP4F2* genes. However, the interaction of clinical factors with the most studied genetic variants of *VKORC1* (-1639G>A), *CYP2C9* (*CYP2C9**2 and *CYP2C9**3) and *CYP4F2* (V433M) impact 56% on warfarin dose variability. In addition to the SNPs mentioned above, this article will also consider other less studied genetic variants, whose impact on the warfarin dose requirement is not as well-known [4].

Table 1.1 shows information about the effect of various polymorphisms on the *VKORC1*, *CYP2C9*, and *CYP4F2* genes on the warfarin dose requirement. The nomenclature used to refer to SNPs may be information about the change in the definition of the SNP nucleotide, e.g. (3730G>A), the possible amino acid change, e.g. (Arg3014His), and refSNP (rs) cluster identification number, e.g. (rs7294). The presentation of Table 1.1 intends to show all the polymorphisms found on the research to assess the SNPs' frequency amongst the European countries. The nomenclature that the various SNPs can present, and their respective names to be used in the study can be found in the "allele name" column.

Table 1.1 - Information regarding the influence that several polymorphisms on the *VKORC1*, *CYP2C9* and *CYP4F2* genes have on the warfarin requirement dose and their different nomenclatures.

Gene	Allele name	Defining Change	Amino acid change	refSNP	SNPs' Effect on warfarin requirement dose
VKORC1	-1639G>A	-1639G>A	-	rs9923231	Reduced warfarin requirement dose
	-1320G>A	-1320G>A	-	rs28533718	High warfarin requirement dose
	-679A>G	-679A>G	-	-	High warfarin requirement dose
	1173C>T	1173C>T	-	rs9934438	Reduced warfarin requirement dose
	2255 T>C	2255 T>C	-	Rs2359612	Reduced warfarin requirement dose
	3730G>A	3730G>A	-	rs7294	High warfarin requirement dose
	6009 C>T	6009 C>T	-	rs17708472	High warfarin requirement dose
	5808T>G	5808T>G	-	rs2884737	Reduced warfarin requirement dose
	6853G>C	6853G>C	-	rs8050894	Reduced warfarin requirement dose
CYP2C9	*CYP2C9**2	430C>T	Arg144Cys	rs1799853	Reduced warfarin requirement dose
	*CYP2C9**3	1075A>C	Ile359Leu	rs1057910	Reduced warfarin requirement dose
CYP4F2	V433M	1297G>A	Val433Met	rs2108622	High warfarin requirement dose
	rs1558139	c.919-446C>T	-	rs1558139	-
	-91T>C	-91T>C	-	rs3093098	-

The *VKORC1* gene located in 16p11.2 is responsible for encoding the Vitamin K epoxide reductase complex, which is the molecular target of warfarin. This gene on chromosome 16 presents several genetic variations, but only the −1639G>A and 1173C>T SNPs have been widely studied. The remaining polymorphisms found in this study are -1320G>A, -679A>G, 6399C>T, 6768G>C, 1173C>T, 2255 T>C, 3730G>A, 6009 C>T, 5808T>G, and 6853G>C [32].

The -1639G>A SNP consists of the substitution of guanine for adenine at position -1639 in the VKORC1's gene upstream promoter region. This gene polymorphism changes the gene transcription, reducing the protein expression by 50%. Hereupon, this SNP will then assign their carriers a lower warfarin resistance, which means they will require a lower dose of this drug. Asians present the highest frequency of this genetic variant, having an A allele frequency in the range of 82% to 96%. The Asians are followed by Caucasians (31%-48%) and Africans (3-15%). The G/G and G/A carriers require a 102% and 52% higher warfarin dose, respectively than the A/A carriers. Thus, only considering this polymorphism's presence, an individual from Africa is more prone to require a higher warfarin dose, while one individual from Asia is more likely to require a lower [55]–[58].

The -1320 G>A SNP, results from a guanine for an adenine substitution, along with the -679 A>G SNP which result of an adenine for a guanine substitution, occur in the 5'-flanking region. Both polymorphisms are more frequent in Jewish individuals but rare amongst other ethnic groups. The homozygous and heterozygous carriers of the A allele show a higher warfarin dose requirement because hese polymorphisms increase the VKORC1 transcription [59], [60].

The 1173 C>T polymorphism is a genetic variance that results from the replacement of cytosine by thymine at position 1173 of intron 1. The C/T, and T/T carriers require an average dose of warfarin of 4.8 mg/day and 3.5 mg/day, respectively. On the other hand, the C/C carriers require a significantly higher average dose of warfarin with 6.2 mg/day. As observed regarding the -1639G> A polymorphism, Asians show a higher frequency for the 1173 C>T polymorphism, accounting for about 90%. Asians are followed by Caucasians (around 41%), and Africans with the lowest T allele frequency (around 7%). Considering the presence of the 1173 C>T polymorphism, one individual from Asia is more likely to require a lower warfarin dose and one individual from Africa is more prone to require a higher [61]–[64].

The 3730 G>A polymorphism is a genetic variance that occurs when there is a substitution of guanine for adenine in the 3' untranslated region of the gene. The presence of this mutant allele means for its carriers an average higher warfarin dose requirement. The A/A carriers demand an average warfarin dose of around 8 mg/day. Conversely, the G/A carriers will demand a warfarin dose in a range of 4.82 mg/day to 5.90 mg/day. The G/G carriers will then require, as expected a considerably lower warfarin dose in a range of 3.36 mg/day to 3.80 mg/day. The A allele frequency is around 32%, and 8%, respectively, in Caucasians, and Asians, while for Africans there is no reported frequency. Therefore, one Caucasian is more prone to require a higher warfarin dose than one individual from Asia. [61], [65].

The 2255 C>T polymorphism is a genetic variance resulting from cytosine to a thymine substitution in the gene's intron 2 region. The T/T carriers require a lower warfarin dose than the C/T carriers and the C/C carriers. The T allele frequency is higher in Asians (around 91%), followed by Caucasians (around 38%), and Africans (around 21%). It is possible to conclude that, one individual from Asia is more prone to require a lower warfarin dose, while one individual from Africa is more likely to demand a higher warfarin dose [66]–[68].

The 6009 C>T SNP is the outcome of the substitution of cytosine to thymine in the intron 1 region of the gene. The presence of this will result in higher resistance to warfarin, increasing the warfarin requirement dose. The Europeans presents the highest frequency of this mutant allele accounting for around 20%. In comparison, Africans and Eastern Asians share the lowest frequency for T allele with 3%. Between these, there are Americans and Southern Asians with a mutant allele frequency of around 14%, and 9%, respectively. Thus, one European individual is more prone to require a higher warfarin dose, and an individual from Southern Asia is more likely to require a lower dose [69], [70].

The 5808 T>G is an intronic *VKORC1* genetic variance consisting of a thymine nucleotide substitution to guanine. The G/G carriers show a lower warfarin dose requirement (about 2.5 mg/day) than G/T heterozygous carriers (about 4.1 mg/day) and T/T carriers (about 6.0 mg/day). The Europeans show the highest G allele frequency (about 26%), followed by Americans (about 18%). On the other hand, Eastern and Southern Asians and Africans show the lowest frequency for this mutant allele with about 0%, 7%, and 1%, respectively. Hereupon, it is possible to verify that one individual from Europe is more prone to require a lower warfarin dose, while one individual from Asia and Africa is more likely to require a higher [71]–[73].

The 6853 G>C SNP is a *VKORC1* intronic variant resulting from a guanine substitution for a cytosine that decreases the warfarin requirement dose. The C/C carriers require a lower warfarin dose than G/C and G/G wild carriers in an unknown proportion. The C allele frequency Eastern Asians is the highest (around 89%), followed by Americans (around 40%) and Europeans (around 44%). On the other hand, Africans and Southern Asians present the lowest C allele frequency with around 25% and 15%, respectively. Considering the presence of this polymorphism, one individual from Eastern Asia is more prone to require a lower warfarin dose, while an individual from Southern Asia is likely to require a higher. [74], [75].

CYP2C9 gene located in 10q23.33 encodes the enzyme responsible for 15% to 20% of the metabolic clearance and S-warfarin metabolism. This enzyme is part of the CYP450 enzyme superfamily, including the CYP2C8, CYP2C18, and CYP2C19 enzymes. It is estimated that 5% to 30% of the world's population carries any *CYP2C9* gene's polymorphism. The *CYP2C9* gene has several polymorphisms that affect warfarin metabolization by promoting or inhibiting it, but in this study, only the *CYP2C9**2 and *CYP2C9**3 polymorphisms will be addressed [55], [76].

The *CYP2C9**2 is the result of cytosine substitution for thymine in the exon 3 region of the *CYP2C9* gene at position 430 leading to an amino acid exchange (Arg144Cys). On the other hand, the *CYP2C9**3 is a 1075 A>C genetic variant in the exon 7 region of the *CYP2C9* gene that will lead to an Ile359Leu amino acid change [55], [77].

Both *CYP2C9**2 and *CYP2C9**3 SNPs are more frequent in Caucasians than in any other, accounting for around 8% to 15% and 6% to 8%, respectively. Africans show a lower frequency for the *CYP2C9**2 (around 0% to 4%) and the *CYP2C9**3 polymorphism (around 0% to 2%). In Asian populations, the *CYP2C9**2 (around 0%) and *CYP2C9**3 (from around 2% to 5%) polymorphisms are also rare. The *1/*2 and *1/*3 carriers will suffer a reduction on the warfarin dose requirement of 19.6% and 33.7%, respectively relatively to *1/*1 carriers. In parallel, *2/*2 and *3/*3 carriers, contrast with an even higher reduction on the warfarin requirement dose of 36.0% and 78.1%, respectively compared to that required by *1/*1 carriers. Finally, the *2/*3 carriers present a 56.7% reduction on the warfarin dose requirement. Therefore, considering the presence of the *CYP2C9**2 and *CYP2C9**3 polymorphisms one Caucasian individual is more prone to require a lower warfarin dose than one Asiatic and African individual [55], [78], [79].

The *CYP4F2* gene located in 19p13.12 encodes for the CYP4F2 protein. This enzyme's importance emerged due to its prominence in catalysing the hydroxylation of VK1's phytyl side chain into hydroxyvitamin K1, which allows the removal of the excess of VK1. The polymorphisms concerning the CYP4F2 gene found during this search were: V433M, -91T>C, and rs1558139 [32].

The V433M is a missense SNP that results from a cytosine substitution to thymine at position 12. The presence of this polymorphism will result on a decrease of the enzyme's catalytic activity, which will increase the levels of VK1 levels, and the warfarin dose requirement in 7%. The Europeans show an T allele's frequency of around 11%, similar to Asiatic (around 9%), while African–American present a much lower frequency (1.4%). Thus, one European individual is more likely to require a higher warfarin dose requirement, while one African-American individual is more prone to require a lower dose [80]–[82].

The genetic variant rs1558139 consists of a substitution of cytosine for thymine in an intronic region. As there are no studies relating the presence of the T allele to the requirement dose of warfarin, there is no information on this subject. [82]–[84].

Regarding the -91T>C SNP, it results of a thymine substitution for a cytosine in the 5' untranslated region of the gene. Its impact on the variability of the warfarin dose requirement as well as its frequency among world's populations, has not been reported [85], [86].

Some genetic polymorphisms may enhance the enzyme's response to warfarin, while others will inhibit it. The use of SNPs on pharmacogenetics studies helps the homogeneity testing, identifying and map complex of common diseases as heart disease and, diabetes. The interest in these polymorphisms intensifies whit the possibility of its large-scale genotyping, enabling its use as a marker to identify genetic variation that predisposes to complex disorders [48]–[50].

2. MATERIAL AND METHODS

2.1. Systematic review

The literature used in this study was found through the search on two article databases - PubMed and Web of Knowledge. The investigation was conducted between July 2020 and August 2020 to find relevant papers about the effect and frequency among European countries of the *VKORC1, CYP2C9,* and *CYP4F2* genes polymorphisms. The keywords used were [*European country*, *gene* and polymorphisms] and [*each European country nationality*, *gene* and polymorphisms] (e.g., [Spain, *VKORC1*, and polymorphisms] and [Spanish, *VKORC1*, and polymorphisms]). The selection criteria were experimental studies and case reports excluding systematic reviews, meta-analysis, and non-written English articles with no restriction on the article's publication year.

For the *VKORC1* gene, there were obtained 327 results after discarding 83 repeated results out of 410. While for the *CYP2C9* gene there were obtained 628 results from which 96 duplicated results were discarded, leaving a total of 532 results. On the other hand, for the *CYP4F2* gene, there were retrieved 123 results, from which 29 results were discarded for a total of 94 available articles. Hereupon, a total of 953 documents obtained from the different searches performed were then scanned to assess the presence of duplicated results, leaving a total of non-duplicated 695 articles (Figure 2.1).

As it is possible to observe in Figure 2.1, after assessing the presence of duplicated articles, they were evaluated and excluded according to their content. The primary excluding criteria were studies in animals (31) while the second exclusion criteria were studies conducted on in- and outpatients (206). The third selection criteria were to exclude studies conducted in healthy control groups whose content does not address the genes under study (309).

Therefore, between the articles studying the *VKORC1*, *CYP2C9* and *CYP4F2* genes polymorphisms in healthy volunteers, 23 articles were excluded for not providing the genotypic distributions of studied controls. Also, seven articles were excluded because they do not provide the allelic frequency of their studied volunteers. The allelic frequencies presented on these studies cannot be obtained through the allele counting from the genotypic distribution since this is presented is grouped.

Hereupon, a total of 576 articles were discarded due to the reasons mentioned above. This leaves a total of 119 validated articles whose content addresses the frequency of the *VKORC1*, *CYP2C9*, and *CYP4F2* genes polymorphisms in healthy European studied populations. Among the validated articles, only the polymorphisms that were mentioned in at least 3 articles were considered for this survey.

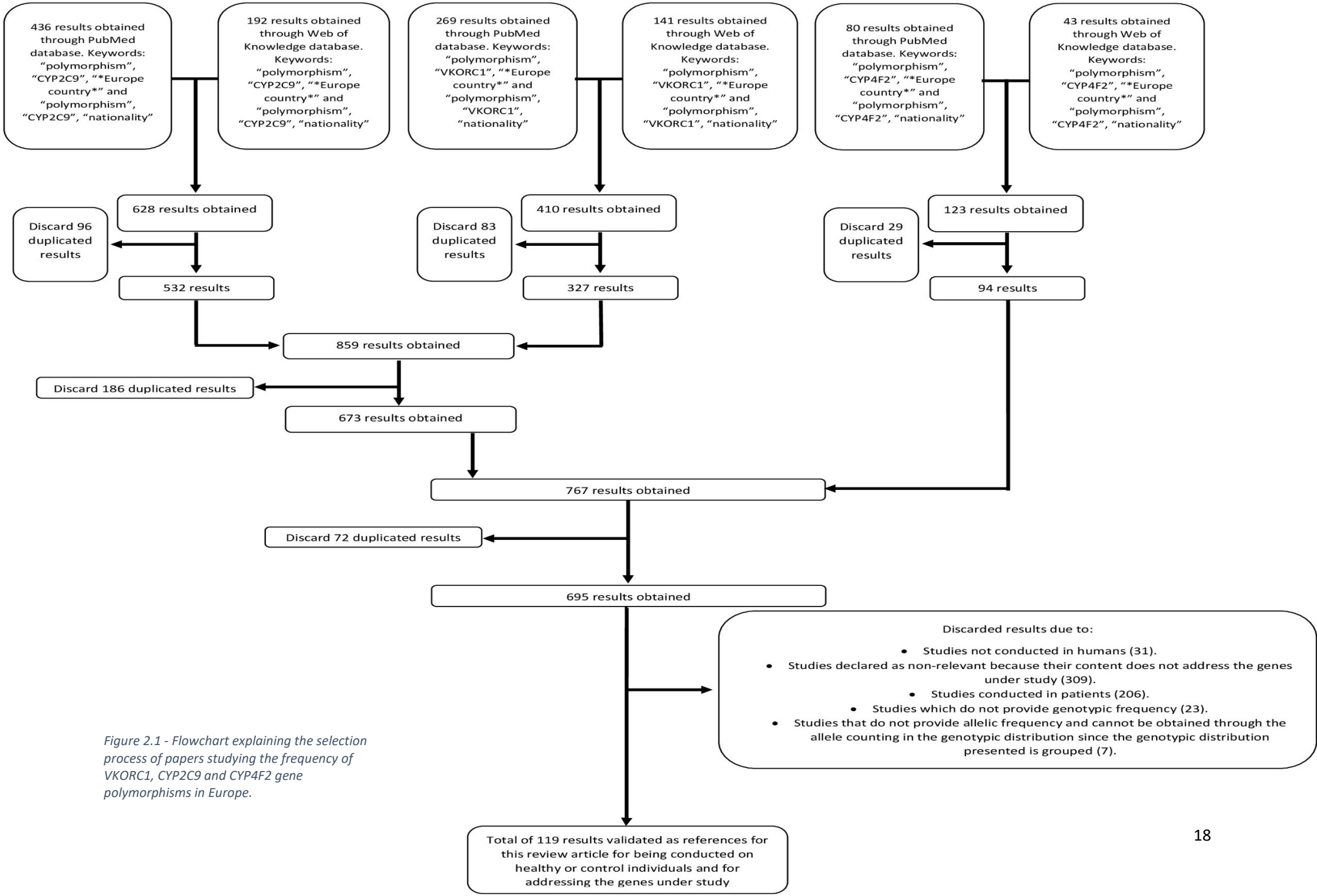

Figure 2.1 - Flowchart explaining the selection process of papers studying the frequency of VKORC1, CYP2C9 and CYP4F2 gene polymorphisms in Europe.

3. DISCUSSION

To facilitate the frequency of polymorphisms' analysis in European countries, these were grouped into six geographical subdivisions according to political and cultural criteria. The referred subdivisions, namely, Northern, Eastern, Southeastern, Central, Southern, and Western Europe, can be observed in Figure 3.1, as well as the respective countries part of them [87]. Although Turkey is not an integral part of any of these subregions, it was considered for study purposes, that this country would be part of the subregion of Southeastern Europe. Concerning Russia, as it is a transcontinental country, only studies in cities/locations within Eastern Europe's delimited area were considered valid.

Figure 3.1 - Geographical subregions of the European continent according to political and cultural criteria. Adapted from [87].

3.1. *VKORC1* –1639G>A gene polymorphism frequency in European countries

A compilation of the genotypic and allelic distribution of the -1639G>A polymorphism among the European countries is presented in Table 3.1. In this research there were validated 36 papers studies published between 2005 and 2020. In the referred studies, there were involved 6911 healthy individuals from 16 countries (Austria, Bosnia, Croatia, Cyprus, France, Germany, Greece, Hungary, Macedonia, Poland, Portugal, Hungary, Romania, Russia, Slovakia, Turkey) across the different European subregions (Figure 3.1). The population's age and gender proportion were not considered because not all studies provide that information. Also, this study only intends to access the polymorphisms' frequency and their impact on the warfarin dose requirement.

Table 3.1 - Genotypic and allelic frequencies for the *VKORC1* -1639G>A gene polymorphism among the European countries							
Population	N	Genotypic Distribution			Allelic Frequency		References
		GG	GA	AA	G	A	
Eastern Europe							
Russian Chuvash (Republic of Chuvashia, Central European Russia)	238	50	137	51	0.498	0.502	[88]
Russian Mari (Republic of Mari El, Eastern European Russia)	206	60	94	52	0.519	0.481	[88]
Russian Kabardian (Republic of Kabardino-Balkaria, Southwestern European Russia)	157	38	90	29	0.529	0.471	[88]
Russian Ossetian (Republic of North Ossetia – Alania, Southwestern European Russia)	244	60	128	56	0.508	0.492	[88]
Central Europe							
Austrian (Vienna, Eastern Austria)	188	68	93	68	0.609	0.391	[89]
Austrian (Graz, Southern Austria)	333	123	161	49	0.611	0.389	[90]
Croatian (Osijek, eastern Croatia)	420	151	196	73	0.593	0.407	[91]
German (Schleswig, Northern Germany)	521	192	243	86	0.602	0.398	[92]
German (Bonn, Western Germany)	200	69	96	35	0.580	0.420	[93]
German (Bonn, Western Germany)	221	80	102	40	0.590	0.410	[94]
German (Bonn, Western Germany)	100	30	53	17	0.560	0.440	[95]
German (Mecklenburg-Vorpommern, Northern Germany)	120	41	58	21	0.580	0.420	[96]
Hungarian (Pecs, Southwestern Hungary)	510	180	262	68	0.610	0.390	[69]
Polish (Warsaw, Eastern Central Poland)	140	61	66	13	0.671	0.330	[97]
Romani (Pecs, Southwestern Hungary)	451	214	206	31	0.703	0.297	[69]
Slovak (Bratislava, Southwestern Slovakia)	112	41	58	13	0.630	0.370	[98]
Southeastern Europe							
Bosnian (Sarajevo, Eastern Bosnia)	129	51	59	19	0.624	0.376	[60]
Greek (Patras, Southwestern Greece)	145	46	70	29	0.559	0.441	[99]

Table 3.1 - Genotypic and allelic frequencies for the *VKORC1* -1639G>A gene polymorphism among the European countries (cont.)							
Population	N	Genotypic Distribution			Allelic Frequency		References
		GG	GA	AA	G	A	
Greek (Ioannina, Northwestern Greece)	82	33	38	11	0.634	0.366	[100]
Greek (Alexandroupolis, Northeastern Greece)	328	109	147	72	0.556	0.444	[101]
Macedonian (Skopje, Northern Central Macedonia)	101	40	46	15	0.624	0.376	[102]
Romanian (Cluj-Napoca, Northwestern Romania)	178	61	92	25	0.601	0.399	[103]
Romanian (Cluj-Napoca, Northwestern Romania)	332	107	170	55	0.578	0.422	[104]
Romanian (Cluj-Napoca, Northwestern Romania)	114	41	47	26	0.566	0.434	[105]
Turkish (Ankara, Northern Central Turkey)	149	40	79	31	0.530	0.471	[106]
Turkish (Gaziosmanpaşa, Northwestern Turkey and Tokat, Northern Central Tukey)	140	47	78	15	0.615	0.385	[107]
Turkish (Gaziosmanpaşa, Northwestern Turkey and Tokat, Northern Central Tukey)	89	22	49	18	0.522	0.478	[108]
Turkish (Gaziosmanpaşa, Northwestern Turkey and Tokat, Northern Central Tukey)	66	14	38	14	0.500	0.500	[109]
Turkish (Kayseri, Central Turkey)	51	19	19	13	0.559	0.441	[110]
Turkish (Eskisehir, Western Central Turkey)	100	22	56	22	0.500	0.500	[111]
Turkish (Eskisehir, Western Central Turkey)	100	22	50	28	0.470	0.530	[112]
Western Europe							
French (Paris, Northern Central France)	222	72	110	40	0.572	0.428	[113]
Southern Europe							
Cypriot (Nicosia, Limasol, Ammochostos, Larnaka, Pafos, and Kerynia, Cyprus)	148	34	70	44	0.466	0.534	[114]
Italian (Lombardia, Northern Italy)	120	49	62	9	0.667	0.333	[115]
Portuguese (Oporto, Northern Portugal	89	30	43	16	0.579	0.422	[116]
Portuguese (São Miguel island, Azores archipelago, Portugal)	58	20	26	12	0.569	0.431	[117]

Analyzing Table 3.1 it is possible to verify that Russia is the only country in Eastern Europe (Figure 3.1) that presents data related to the presence of -1639G>A polymorphism in its population. A total of 856 healthy individuals spread across the Republics of Chuvashia, Mari El, Kabardino-Balkaria, and North Ossetia-Alania, respectively belonging to the Chuvash, Mari, Kabardian and Ossetian ethnic groups had their genotypic profile assessed.

The 238 Chuvash volunteers presented the highest G/A carriers' frequency with 57.6%, and a frequency for the A/A carriers of 21.4%. Such genotypic distribution resulted in the highest A allele frequency with 50.2%. The 157 genotyped Kabardin individuals presented the lowest frequency for the A/A carriers with 18.5%, and a G/A carriers' frequency of 57.3%. This genotypic distribution resulted in an A allele of 47.1% [88].

Furthermore, the 206 genotyped Mari volunteers presented the highest frequency for A/A carriers (25.5%) and the lowest for G/A carriers (45.6%), resulting on an A allele frequency of 48.1%. Finally, the 244 genotyped Ossetians presented a frequency of 23.0%, and 24.6%, respectively for A/A and G/A carriers, resulting in an A allele frequency of 49.2% [88].

Hereupon, it is possible to predict which individual from the different Russian studied populations is more prone to require a higher warfarin dose. One individual from the Republic of Chuvashia is more prone to present a lower warfarin dose requirement. This occurs since the studied Chuvash population presented the highest A allele frequency with 50.2%. On the other hand, one individual belonging to the Kabardian ethnic group is more likely to require a higher warfarin dose. This happens because the studied Kabardian displayed the lowest A allele frequency with 47.1%. It is important to note that these predictions only consider the presence and influence of the -1639 G>A polymorphism [56], [88].

The -1639G>A polymorphism's frequency assessment in Central European countries (Figure 3.1) involved 3316 volunteers from 6 different countries (Austria, Croatia, Germany, Hungary, Poland, and Slovakia) spread across 11 study populations (Table 3.1).

The two studies evaluating the -1639G>A polymorphism's genotypic distribution in the Austrian population genotyped 188 volunteers from Vienna [89] and 333 from Graz [90]. The genotyped individuals from Vienna, and Graz showed a G/A carriers' frequency of 49.5% and 48.3%, respectively. Moreover, for the genotyped volunteers from Vienna and Graz the A/A carriers' frequency was 14.4% and 14.7%, respectively. This genotypic distribution resulted in an A allele frequency of 39.1%, and 38.9%, for populations from Vienna, and Graz, respectively. The presented similarity for the A allele frequency is understandable and explicable since these cities are only 200 km apart from each other.

Five papers contain information about the -1639G>A polymorphism's genotypic distribution in 1162 individuals from 3 different cities in Northern and Western Germany. The involved volunteers under study were from Bonn [93]–[95], Schleswig [92], and Mecklenburg-Vorpommern [96].

Three studied populations from Bonn had their genotypic profiles assessed. The 221 studied individuals by Watzka et al. [94] showed a G/A, and A/A carriers' frequency of, respectively, 46.6%, and 18.1%. Such genotypic distribution resulted in the lowest A allele frequency with 41.0%. Furthermore, the 100 studied individuals by Spohn et al. [95] presented a frequency for the G/A and A/A carriers of, respectively, 53.0%. and 17.0%, which resulted in an A allele frequency of 44.0%. Finally, the 200 controls assessed by Geisen et al. [93] displayed a G/A, and A/A carriers' frequency of 48.0%, and 17.5%, accounting for an A allele frequency of 42.0%.

Hereupon, it is possible to note that the studied population by Spohn et al. [95] show a disparate genotypic distribution when compared to the other Bonn's populations [93], [94]. Such differences suggest a bias on the population sampling that skew the allelic frequencies. Therefore, these individuals will not be considered in further analysis for not representing the Bonn's population.

The 120 volunteers from Mecklenburg-Vorpommern [96] showed a G/A (48.3%), and A/A (17.5%) carriers' frequency that accounts for an A allele frequency of 41.7%. Similarly, the 521 volunteers from Schleswig [92] displayed a G/A (46.6%), and a A/A (16.5%) carriers' frequency that resulted in a comparable A allele frequency of 39.8%. These presented A allele frequencies are, as expected, very similar to those presented by the studied populations from Bonn [94], [95].

France is the only country representing the Western European (Figure 3.1, Table 3.1) countries. The 222 studied individuals from Paris [113] showed a G/A and a A/A carriers' frequency, respectively of 49.5%, and 18.0%, which accounts for an A allele frequency of 42.8%. This presented A allele frequency compares to those presented in the genotyped populations from Bonn studied by Geisen et al. [93] (42.0%) and Watzka et al. [94] (41.0%), Schleswig [92] (39.8%), and Mecklenburg-Vorpommern [96] (41.7%). Such similarities were expected as France and Germany are neighbouring countries.

The study conducted in Hungary had as controls two populations living in Pecs. These studied populations involved 510 native Hungarians, and 451 Romani living in Pecs [69].

The studied Romani populations presented frequencies for the G/A (45.7%) and A/A (6.9%) carriers that resulted in an A allele frequency of 29.7%. Conversely, the Hungarian genotyped controls presented a higher frequency for the G/A, and A/A carriers with, respectively, 51.4%, and 13.3%. The genotypic distribution found in the studied Hungarians resulted in an A allele frequency of 39.0%. Such disparities in the A allele frequency between these populations may be due to Romani's strict marriage tradition, where marrying someone who is not from the Roma community is not welcomed [69].

The 140 Polish studied individuals [97] presented a G/A (47.1%), and A/A (9.3%) carriers' frequency that resulted in an A allele frequency of 33.0%. Moreover, the 420 genotyped Croatians [91] showed a frequency for the G/A, and A/A carriers' of 46.7%, and 17.4%, respectively. This genotypic distribution results on an A allele frequency of 40.7% for Croatians. Also, the 112 assessed Slovaks [98] showed a frequency for the G/A (51.8%), and a A/A (11.6%) carrier's frequency that resulted in in an A allele frequency of 37.0%.

The studied Slovaks [98] and Polish [97] showed a comparable A allele frequency, which is understandable since these are neighbouring countries. Moreover, the A allele frequency found in the studied population from Croatia [91] resemble those presented by the genotyped individuals from Bonn studied by Geisen et al. [93] (42.0%) and Watzka et al. [94] (41.0%), Schleswig [92] (39.8%), Mecklenburg-Vorpommern [96] (41.7%) Viena [89] (39.1%), Graz [90] (38.9%), Hungary (native individuals) [69] (39.0%), and France [113] (42.8%). Also, for this case the similar A allele frequencies are explainable because of geographical proximity.

The studied Romani [69] and Germans from Mecklenburg-Vorpommern [96] then accounted, respectively, for the lowest (29.7%) and highest (42.0%) A allele frequency amongst the studied Central European populations. Therefore, it is possible to note that one Romani individual is more prone to require a higher warfarin dose. Conversely, one individual from Mecklenburg-Vorpommern is more likely to require a lower warfarin dose. It is important to note that these predictions only consider the presence and influence of the -1639 G>A polymorphism [56].

In the research carried out to evaluate the -1639G>A polymorphism's genotypic distribution in Southeastern Europe (Figure 3.1) a total of 15 articles were obtained. These papers assessed 2113 individuals from 5 different countries, namely Bosnia and Herzegovina, Greece, Macedonia, Romania, and Turkey (Table 3.1).

In Greece, three populations from the southeast, northwest, and northeast had their genotypic profile for the -1639G>A polymorphism assessed. The 82 studied individuals from Ioannina [100] showed a G/A, and A/A carriers' frequency of, respectively, 46.3%, and 13.4%. The genotypic distribution presented by genotyped controls from Ioannina resulted in the lowest A allele frequency amongst the Greek populations with 36.6%.

The geographical proximity of the city of Ioannina with Macedonia and Bosnia explains the similar A allele frequency found between their populations [60], [100], [102]. The 129 genotyped Bosnian [60] and the 101 studied Macedonian [102] showed a concordant frequency for the G/A (45.7%, and 45.5%, respectively), and A/A (45.5%, and 14.9%, respectively) carriers. This genotypic distribution also resulted in a similar A allele frequency for the Bosnian (37.6%) and Macedonian (37.6%) genotyped volunteers.

On the other hand, the studied 145 controls from Patras [99] and the 328 volunteers from Alexandroupolis [101] presented very a similar frequency either for the G/A (48.3%, and 44.8%, respectively), and A/A (20.0%, and 22.0%, respectively) carriers. Such a similar genotypic distribution resulted in a comparable A allele frequency for the studied populations from Patras (44.1%) and Alexandroupolis (44.4%).

In Romania, three studies [103]–[105] conducted in 624 healthy volunteers from Cluj-Napoca assessed their -1639G>A polymorphism's genotypic distribution. The 178 assessed individuals by Groza et al. [103] showed a G/A and a A/A carriers' frequency of, respectively, 51.7%, and 14.0%. Such genotypic distribution resulted in an A allele frequency of 39.9%. Moreover, the 332 studied volunteers by Buzoianu et al. [104] showed a similar frequency for the G/A (51.2%), and A/A (16.6%) carriers with that presented by the individuals studied by Groza et al. [103]. The presented genotypic distribution then resulted in an A allele frequency of 42.2%.

On the other hand, the 114 genotyped individuals by Vesa et al. [105] showed discrepant frequencies for the G/A (41.2%) and A/A (22.8%) carriers with other Cluj-Napoca studied populations [103], [104]. This genotypic distribution in an A allele frequency of 43.4%. Such differences suggest a bias on the population sampling that skew the genotypic distribution. Therefore, the individuals studied by Vesa et al. [105] will not be considered in further analysis for not representing the Cluj-Napoca's population.The studied populations from Romania [103], [104] show a similar A allele frequency with populations from neighboring countries, namely genotyped Hungarians (native individuals) [69] (39.0%), Slovaks [98] (37.5%), Bosnian [60] (37.6%), Croatians [91] (40.7%), and Macedonian [102] (37.6%).

The seven studies obtained addressing the -1639G>A polymorphism's genotypic distribution in the Turkish populations involved 695 individuals. The genotyped controls were spread over five study populations from the cities of Ankara, Gaziosmanpaşa, Tokat, Kayseri and Eskisehir.

Three of the seven referred papers portray the genotypic distribution in grouped study populations from cities of Gaziosmanpaşa and Tokat. As the number of studied individuals from each city is not showed in the studies, it is not possible to compare the genotypic distribution of each studied population from Gaziosmanpaşa and Tokat [107]–[109].

The 89 genotyped controls by Demir et al. [108] showed a G/A, and a A/A carriers' frequency of, respectively, 55.1%, and 20.2%, resulting in an A allele frequency of 47.8%. Simultaneously, the 66 assessed individuals by Ortak et al. [109] showed similar G/A (57.6%) and A/A (21.2%) carriers' frequencies, accounting for an A allele frequency of 50.0%.

On the other hand, the 140 evaluated individuals by Kutluturk et al. [107] displayed a G/A, and A/A carriers' frequency, of respectively, 55.7%, and 10.7%, accounting for an A allele frequency of 38.5%. Such differences with the remaining studied individuals from Gaziosmanpaşa-Tokat [108], [109] suggest a bias on the population sampling that skew the allelic frequencies. Therefore, the individuals studied by Kutluturk et al. [107] will not be considered in further analysis for not representing the combined populations of Gaziosmanpaşa and Tokat.

There were genotyped a total of 200 individuals from Eskisehir divided in two study populations [111], [112]. The 100 evaluated individuals studied by Ozbayer et al. [111] showed a frequency for the G/A, and A/A carriers, respectively of 56.0%, and 22.0%. This genotypic distribution will then result in an A allele frequency of 50.0%. Conversely, the 100 assessed individuals by Cosan et al. [112] displayed a G/A and A/A carriers' frequency of 50.0%, and 28.0%, accounting for an A allele frequency of 53.0%.

The genotypic distributions' differences among the two studied populations from Eskisehir, suggests that their genotypic distributions are not comparable [111], [112]. As Ankara is about 250 km apart from Eskisehir, it is expected that the genotypic distribution among their populations does not differ much. The 149 individuals from Ankara studied by Taşkın et al. [106] displayed a G/A (53.0%) and A/A (20.8%) carriers' frequency, resulting on an A allele frequency of 47.1%. Therefore, it is possible to verify that the studied population from Ankara showed a more comparable genotypic distribution with the studied individuals by Ozbayer et al. [111]. Conversely, the assessed individuals by Cosan et al. [112] will not be considered in further analysis for not representing the population of Eskisehir.

The 51 studied individuals from Kayseri [110] showed a frequency for the G/A, and A/A carriers of respectively, 37.3%, and 25.5%, representing an A allele frequency of 44.1%. The notorious difference in this population's allelic frequencies with the other Turkish populations, suggests that there was a sampling error. Therefore, the studied population from Kayseri will not be considered in further analysis.

The studied Turkish individuals from Gaziosmanpaşa-Tokat [108], [109], Eskisehir [111], and Ankara [106] showed a similar A allele frequency with the studied Greeks from Alexandroupolis [101] (44.4%) and Patras [99] (44.1%). This happens because Turkey is geographically close, and due to the Turkish preponderant influence in the region.

Also, the referred studied populations from Turkey present a similar A allele frequency with the genotyped Russians from the Republics of Chuvashia (50.2%), North Ossetia-Alania (49.2%), Mari El (48.1%), Kabardino-Balkarian (47.1%) [118].

The studied population from Ioannina [100] and Eskisehir [111] accounted, respectively for the lowest (36.6%) and highest (50.0%) A allele frequency amongst the Southeastern European countries. Then it is possible to predict that one individual from Ioannina is more likely to require a higher warfarin dose than one person from Eskisehir. It is important to note that these predictions only consider the presence and influence of the -1639 G>A polymorphism [56].

In the Southern European (Figure 3.1) countries, four studies involving 415 healthy volunteers assessed their -1639G>A polymorphism's genotypic profile. The studied populations were spread across three different countries, namely Cyprus, Italy and, Portugal (Table 3.1).

The 58 studied islanders from São Miguel [117] showed a G/A, and a A/A carriers' frequency of 44.8%, and 20.7%, respectively, which represented an A allele frequency of 43.1%. Simultaneously, the 89 genotyped individuals from Oporto [116] showed similar frequencies for the G/A (48.3%) and A/A (18.0%) carriers, accounting for an A allele frequency of 42.2%. Such similarities on these A allele frequencies would be expected since these are populations from the same country.

The 148 genotyped volunteers from Cyprus [114] displayed a G/A, and a A/A carriers' frequency respectively of, 47.3%, and 29.7%. This genotypic distribution then represented an A allele frequency of 53.4%. The A allele frequency presented by the studied Cypriots is comparable with that presented by the genotyped Turkish from Gaziosmanpaşa-Tokat studied by Demir et al. [108] (47.8%), and Ortak et al. [109] (50.0%), Eskisehir [111] (50.0%), and Ankara [106] (47.1%). This happens due to the strong Turkish influence in Cyprus, as well as the geographical proximity.

On the other hand, the 120 studied Italians from Lombardia [115] showed a frequency for the G/A, and A/A carriers, respectively of 51.7%, and 7.5%. Such genotypic distribution accounted for an A allele frequency of 33.3%. The region of Lombardia is located in Northern Italy, which is geographically close to Central European and Balkan countries. This results in similar A allele frequencies between the individuals from Lombardia and populations from Poland [97] (32.9%), Ioannina [100] (36.6%), Slovakia [98] (37.5%), Bosnia [60] (37.6%), and Macedonia [102] (37.6%).

The studied populations that showed the highest and lowest A allele frequency are, respectively Cypriot [114] (53.4%) and Romani [69] (29.7%). These two studied populations are necessarily isolated from other populations since the Cypriots are islanders and the Romani do not usually homogenize with the environment in which they are inserted. Thus, this favours the conservation of their genetic code, making them present such disparate frequencies for the A allele compared to other populations.

The studied population from Italy [115] accounted for the lowest A allele frequency (33.3%) amongst the Southern European populations, while the studied Cypriots [114] for the highest (53.4%). Therefore, the one individual from Lombardia is more prone to require a higher warfarin dose. Conversely, one person from Cyprus, has a higher probability to require a lower warfarin dose. It is important to note that these predictions only consider the presence and influence of the -1639 G>A polymorphism [56].

3.2. *VKORC1* 1173C>T gene polymorphism frequency in European countries

Nine studies published between 2005 and 2019 were validated to assess the 1173C>T polymorphism's presence in European populations. The 3030 assessed individuals were spread across Central, Southeastern, Western, and Southern European countries (Figure 3.1, Table 3.2). As this study only intends to access the polymorphism's impact on the warfarin dose requirement, the population's age and gender were not considered.

Table 3.2 - Genotypic and allelic frequencies for the *VKORC1* 1173C>T gene polymorphism among the European countries							
Population	N	Genotypic Distribution			Allelic Frequency		References
		CC	CT	TT	C	T	
Central Europe							
Croatian (Osijek, eastern Croatia)	420	151	196	73	0.593	0.407	[91]
Slovak (Bratislava, Southwestern Slovakia)	112	41	58	13	0.630	0.370	[98]
Southeastern Europe							
Romanian (Cluj-Napoca, Northwestern Romania)	136	45	55	36	0.533	0.467	[119]
Turkish (Gaziosmanpaşa, Northwestern Turkey and Tokat, Northern Central Tukey	89	22	49	18	0.522	0.478	[108]
Turkish (Gaziosmanpaşa, Northwestern Turkey and Tokat, Northern Central Tukey	66	14	38	14	0.500	0.500	[109]
Western Europe							
French (Paris, Northern Central France)	24	5	12	7	0.458	0.542	[120]
French (Brest, Northwestern France)	439	158	210	71	0.600	0.400	[121]
Dutch (General population, Netherlands)	1667	654	762	251	0.621	0.379	[122]
Southern Europe							
Italian (Naples, Southern Italy)	77	20	45	12	0.552	0.448	[123]

In France (Western Europe) (Figure 3.1), two studied populations from Brest [121] and Paris [120] were assessed for the 1173C>T polymorphism's frequency. The 439 studied individuals from Brest showed a C/T, and T/T carriers' frequency of, respectively, 47.8%, and 16.2%. The T allele frequency that resulted from the referred genotypic distribution was 40.0%. Conversely, the 24 genotyped volunteers from Paris presented a frequency for the C/T (50.0%), and T/T (29.2%) carriers, resulting in a T allele frequency of 54.2%.

The 1667 assessed Dutch [122] (Netherlands, Western Europe) (Figure 3.1) presented a C/T (45.7%), and T/T (15.1%) carriers' frequency that resulted in a T allele frequency of 37.9%. Therefore, it is possible to verify a disparate T allele frequency in the studied population from Paris (54.8%) [120] compared to the genotyped individuals from Brest (40.0%) [121] and the Netherlands (37.9%). Hereupon, the study conducted in individuals from Paris for presenting a small sample size cannot be representative of the population of Paris and will be excluded from further analysis.

The 420 genotyped Croatians from Osijek [91] (Central Europe) (Figure 3.1) showed a C/T, and T/T carriers' frequency, respectively of 46.7%, and 17.4%. The T allele frequency for the studied Croatians accounted for 40.7%. Moreover, the 112 studied Slovaks [98] (Central Europe) (Figure 3.1) presented a C/T (51.8%), and T/T (11.6%) carriers' frequency that resulted in a T allele frequency of 37.0%. Therefore, it is possible to verify that the studied individuals from Slovakia and Croatia showed a comparable T allele frequency with that presented by the assessed French from Brest (40.0%) [121] and Dutch (37.9%) [122]. The reason for these similar T allele frequencies may lie on the geographical proximity.

In Turkey (Southeastern Europe), two grouped populations from Gaziosmanpaşa and Tokat had their genotypic profile assessed in two studies. As the number of individuals from each city is not available in the papers, it is impossible to dissociate the two populations of Gaziosmanpaşa and Tokat [108], [109].

The 89 volunteers from Gaziosmanpaşa and Tokat studied by Demir et al. [108] showed a C/T, and T/T carriers' frequency respectively of 55.1%, and 20.2%. The T allele frequency resulted in 47.8% from the presented genotypic distribution. Simultaneously, the 66 genotyped controls by Ortak et al. [109] showed frequencies for the C/T (57.6%), an T/T (21.2%) carriers, resulting on a comparable T allele frequency of 50.0%.

The 77 individuals from Naples in Italy [123] (Southern Europe) displayed a C/T (58.4%), and T/T (15.6%) carriers' frequency that resulted in a T allele frequency of 44.8%. Simultaneously, the 136 studied individuals from Romania [119] (Southeastern Europe) showed a C/T, and a T/T carriers' frequency, respectively of 40.4%, and 26.5%. This genotypic distribution resulted in a similar T allele frequency of 46.7% with that presented by the studied Italians. Also, the Turkish populations studied by Ortak et al. [109], and Demir et al. [108] showed a comparable T allele frequency (respectively with 50.0%, and 47.8%) compared with the genotyped Romanians and Italians. The similar T allele frequencies for these studied populations may be due to the geographical proximity.

The genotyped Slovaks [98] and the studied individuals by Ortak et al. [109] from Gaziosmanpaşa-Tokat presented, respectively the lowest (37.0%) and highest (50.0%) T allele frequency. Therefore, it is possible to verify that a Slovak individual is more prone to require a higher warfarin dose. On the other hand, one Turkish individual presents the highest probability to require a lower warfarin dose. It is important to note that these predictions only consider the presence and influence of the 1173C>T polymorphism [63], [64].

3.3. VKORC1 3730G>A gene polymorphism frequency in European countries

The data regarding the 3730 G>A polymorphism presence in different European populations from Central, Southeastern, and Southern Europe (Figure 3.1) is presented in Table 3.*3*. Six papers assessed the genotypic and allelelic frequency of this polymorphism in 1362 healthy individuals spread across four countries (Hungary, Portugal, Slovakia, and Turkey). Information as age or gender was not considered as the aim of this study is only to access the 3730G>A frequency in the populations.

Table 3.3 - Genotypic and allelic frequencies for the VKORC1 3730G>A gene polymorphism among the European countries

Population	N	Genotypic Distribution			Allelic Frequency		References
		GG	GA	AA	G	A	
Central Europe							
Hungarian (Pecs, Southwestern Hungary)	510	206	233	71	0.632	0.368	[69]
Romani (Pecs, Southwestern Hungary)	451	132	220	99	0.537	0.463	[69]
Slovak (Bratislava, Southwestern Slovakia)	112	43	47	22	0.590	0.410	[98]
Southeastern Europe							
Turkish (Eskisehir, Western Central Turkey)	100	37	53	10	0.635	0.365	[111]
Turkish (Eskisehir, Western Central Turkey)	100	41	40	19	0.610	0.390	[112]
Southern Europe							
Portuguese (Oporto, Northern Portugal	89	44	40	5	0.714	0.286	[116]

Two studies carried out in Turkish individuals from Eskisehir [111], [112] (Southeastern Europe) (Figure 3.1) involved 200 volunteers. The 100 individuals studied by Ozbayer et al. [111] displayed a G/A (53.0%), and A/A (10.0%) carriers' frequency that resulted in an A allele frequency of 36.5%. On the other hand, the 100 volunteers studied by Cosan et al. [112] presented a frequency for the G/A, and A/A carriers, respectively of 41.0%, and 40.0%. This genotypic distribution result on an A allele frequency of 39.0%. Despite both studied populations presented similar A allele frequencies these also presented disparate genotypic distributions. This suggests that at least one of these studied populations do not represent the Eskisehir's population. Therefore, because there is no other Turkish studied population that can serve as a term of comparison, neither of these populations will be included in further analysis.

The 451 studied Romani [69] living in Hungary (Central Europe) (Figure 3.1) showed a G/A (48.8%), and A/A (22.0%) carriers' frequency that resulted in an A allele frequency of 46.3%. On the other hand, the 510 genotyped Hungarians [69] (Central Europe) (Figure 3.1) presented a frequency for the G/A, and A/A carriers, respectively of 45.7%, and 13.9%. This genotypic distribution resulted in an A allele frequency of 36.8%.

The 112 genotyped Slovaks [98] (Central Europe) showed a frequency for the G/A (42.0%), and A/A (19.6%) carriers', resulting in an A allele frequency of 41.0%. The similar A allele frequency presented by the studied populations from Slovakia and Hungary [69] (36.8%) is understandable since they are neighboring countries.

The 89 studied Portuguese [116] (Southern Europe) showed disparate frequencies for the G/A (44.9%), and A/A (5.6%) carriers with the remaining studied European populations. This genotypic distribution then resulted in the lowest A allele frequency with 28.1%.

Thus, the Romani [69] and Portuguese [116] studied populations showed, respectively, the highest (46.3%) and lowest (28.6%) A allele frequency. This means that one Portuguese individual is more likely to require a lower warfarin dose than one Romani individual. This prediction only considers the presence and influence of the 3730G>A polymorphism [65].

3.4. *CYP2P9**2 and *CYP2C9**3 polymorphisms frequency in European countries

The *CYP2C9**2 and *CYP2C9**3 polymorphisms' frequency in different European populations can be consulted in Table 3.4. The 136 articles found contained information for these polymorphisms in 120667 healthy individuals across 28 different countries spread in the six different European subdivisions (Figure 3.1).

The articles found differed in the approach regarding the genotypic survey of studied populations. Some papers assessed the homozygous carriers' frequency of wild (*1/*1) and mutant (*2/*2; *3/*3) alleles. Also, the frequency for the carriers of wild type allele/mutant allele (*1/*2; *1/*3) and mutant allele/mutant allele (*2/*3) genotypes was evaluated in these populations. Other paper only presented the grouped number of carriers of wild type/mutant, and mutant/mutant genotypes (*1/*2, *1/*3, *2/*2, *2/*3, and *3/*3), as is the case of the British volunteers from Tayside and Fife [124]. These studied populations can be then denominated fully genotyped populations.

. Moreover, some populations only presented the grouped number of carriers for some genotypes. By a way of example, one studied population from Zagreb [125] assessed for the grouped number of the carriers of the *1/*2, and *1/*3 genotypes as well as the carriers of the *2/*2, and *3/*3 genotypes.

On the other hand, other studies separately evaluate the presence of the *CYP2C9**2 and *CYP2C9**3 polymorphisms. These populations are then studied for the *1/*2 and *2/*2 genotypes' presence separately concerning the *1/*3, and *3/*3 genotypes. Thus, the presence of the *2/*3 genotype was not evaluated in these populations. Some other studied populations also do not have some genotypes assessed, as is the case of the studied individuals from Brussels [126] and Istanbul [106] that only do not show information for the *2/*2 genotype.

The non-fully genotyped populations will suffer an excess deviation on the *1 allele frequency compared to the fully genotyped populations. This occurs because the alleles present in a particular non-evaluated genotype will be counted as being the *1 allele. Taking this into account, the frequency values for the homozygous wild-type (*1/*1), and wild-type/ mutant (*1/*2, *1/*3) carriers will be skewed, and consequently the *2 and *3 alleles frequency. By way of example, in the case of populations only assessed for the *1/*1, *1/*2, and *2/*2 genotypes' presence, the *3 allele frequency will be counted as being the *1 allele. This skew of the genotypic distributions occurs for every population that do not have any of the *1/*2, *2/*2, *2/*3, *1/*3, and *3/3 genotypes assessed.

Hereupon, the comparison among populations regarding the *2 and *3 allele frequencies can only be done for the fully genotyped populations. Also, a proper prediction and comparison of the warfarin dose requirement can also only be assessed among the fully genotyped populations. This happens because their genotypic profile regarding the *CYP2C9**2 and *CYP2C9**3 polymorphisms was fully assessed, including the *2/*3 genotype.

Table 3.4 - Genotypic and allelic frequencies for the *CYP2C9**2 and *CYP2C9**3 polymorphisms among the European countries.											
Population	N	Genotypic Distribution						Allelic Frequency			References
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
Eastern Europe											
Russian Balkar (Kabardino-Balkarian Republic, Southwestern European Russia)	200	153	-	-	-	43	4	0.873	-	0.128	[127]
		149	47	4	-	-	-	0.863	0.138	-	
Russian Tatar (Republic of Tatarstan, Eastern European Russia)	141	117	-	-	-	18	6	0.894	-	0.106	
	140	118	21	1	-	-	-	0.911	0.082	-	
Russian (Bashkortostan Republic, Eastern European Russia)	133	109	-	-	-	24	0	0.910	-	0.090	[128]
	112	95	17	0	-	-	-	0.924	0.076	-	
Russian Tatar (Bashkortostan Republic, Eastern European Russia)	129	115	-	-	-	14	0	0.946	-	0.054	
	107	96	11	0	-	-	-	0.949	0.051	-	
Russian Bashkir (Bashkortostan Republic, Eastern European Russia)	65	57	-	-	-	8	0	0.938	-	0.062	
	57	50	7	0	-	-	-	0.939	0.061	-	
Russian Avar (Dagestan, Southern European Russia)	90	65	-	-	-	22	3	0.844	-	0.156	[129]
Russian Lak (Dagestan, Southern European Russia)	46	31	-	-	-	12	3	0.805	-	0.195	
Russian Dargin (Dagestan, Southern European Russia)	50	34	-	-	-	15	1	0.840	-	0.160	
Russian (Moscow, Central European Russia)	352	243	73	2	7	26	1	0.831	0.119	0.050	[130]
Russian (Moscow, Central European Russia)	177	128	28	-	1	20	0	0.859	0.082	0.059	[131]
Russian (Voronezh, Central European Russia)	290	19	53	2	4	33	1	0.828	0.105	0.067	[132]
Russian Sámi (Kola Peninsula, Northern European Russia)	49	41	-	-	-	8	0	0.918	-	0.082	[133]
Russian Mari (Mari El Republic, Eastern European Russia)	206	182	24	0	-	-	-	0.942	0.058	-	[88]
		161	-	-	-	42	3	0.883	-	0.117	
Russian Kabardian (Kabardino-Balkarian Republic, Southwestern European Russia)	157	122	33	2	-	-	-	0.882	0.118	-	
		114	-	-	-	41	2	0.857	-	0.143	
Russian Ossetian (North Ossetia – Alania Republic, Southwestern European Russia)	244	212	31	1	-	-	-	0.932	0.068	-	
		186	-	-	-	52	6	0.869	-	0.131	
Northern Europe											
Danish (Faroe Islands archipelago, Denmark)	311	228	50	0	5	28	0	0.859	0.088	0.053	[134]

Table 3.4 - Genotypic and allelic frequencies for the *CYP2C9**2 and *CYP2C9**3 polymorphisms among the European countries.(cont.).

Population	N	Genotypic Distribution						Allelic Frequency			References
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
Danish (general population)	10392	7996	2232	164	-	-	-	0,877	0,123	-	[135]
		9088	-	-	-	1262	42	0,936	-	0,064	
	36856	28353	7914	589	-	-	-	0,877	0,123	-	
		32284	-	-	-	4442	130	0,936	-	0,064	
Danish (Odense, Southern Denmark)	276	190	53	4	6	23	0	0.826	0.121	0.053	[136]
Danish (Copenhagen, Southeastern Denmark)	175	117	39	4	3	11	1	0.780	0.143	0.046	[137]
Finnish (Helsinki, Southwestern Finland)	71	61	-	-	-	10	1	0,923	-	0,077	[133]
Finnish (Helsinki, Southwestern Finland)	29	17	8	0	1	3	0	0,776	0,155	0,069	[138]
Finnish (Helsinki, Southwestern Finland)	12	9	1	0	1	1	0	0,833	0,083	0,083	[139]
Finnish (Turku, Southwestern Finland)	447	347	94	6	-	-	-	0,881	0,119	-	[140]
	444	388	-	-	-	54	2	0,935	-	0,065	
Norwegian (Oslo, Southern Norway)	309	213	55	2	2	36	1	0,837	0,099	0,065	[134]
Swedish (Stockholm, Southeastern Sweden)	22	6	3	3	4	5	1	0,455	0,295	0,25	[141]
Swedish (Huddinge, Southeastern Sweden)	430	287	80	2	8	50	3	0,819	0,107	0,074	[142]
Swedish (Stockholm, Southeastern Sweden)	1503	1025	269	21	18	165	5	0,83	0,11	0,065	[143]
Central Europe											
Austrian (Vienna, Eastern Austria)	375	244	79	7	3	40	2	0.809	0.128	0.063	[144].
Croatian (general population)	177	122	41	12	1	1	0	0.839	0.124	0.037	[145]
Croatian (general population)	1080	645	254	19	21	138	3	0.779	0.145	0.076	[146]
Croatian (Zagreb, Northern Croatia)	52	40	11	1	-	0	-	0.875	0.125	0.000	[147]
Population	N	*1/*1		*1/*2, *1/*3		*2/*2, *3/*3		*1	*2	*3	References
Croatian (Zagreb, Northern Croatia)	200	148		45		7		0.740	0.165	0.095	[125]
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
Czech (Prague, Northwestern Czech Republic)	254	170	51	5	1	25	2	0.819	0.122	0.059	[148]
German (General population, Germany)	675	599	-	-	-	74	2	0,940	-	0,060	[149]
	673	524	137	12	-	-	-	0,880	0,120	-	

Table 3.4 - Genotypic and allelic frequencies for the *CYP2C9**2 and *CYP2C9**3 polymorphisms among the European countries.(cont.).											
Population	N	Genotypic Distribution						Allelic Frequency			References
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
German (Berlin, Northeastern Germany)	309	205	66	5	6	24	3	0,809	0,133	0,058	[150]
German (Münster, Northwestern Germany)	66	43	8	1	4	10	0	0,796	0,140	0,064	[151]
German (Bonn, West Central Germany)	107	91	-	-	-	12	4	0,910	-	0,090	[95]
	105	79	22	4	-	-	-	0,870	0,130	-	
German (Würzburg, Central Germany)	283	181	52	5	5	39	1	0,800	0,118	0,081	[152]
Hungarians (General population, Hungary)	535	332	104	11	8	74	6	0,787	0,125	0,088	[153]
Polish (Łódź, Central Poland)	97	97	-	-	-	0	0	1,000	-	0,000	[154].
		76	21	0	-	-	-	0,892	0,108	-	
Polish (Warsaw, Eastern Central Poland)	114	71	18	0	5	20	0	0,781	0,096	0,105	[97]
Polish (Poznań, Western Central Poland)	30	21	2	-	-	7	-	0,850	0,033	0,117	[155]
Slovak (Bratislava, Southwestern Slovakia)	112	77	16	2	3	14	0	0,831	0,119	0,050	[156]
Swiss (Bern, Northwestern Switzerland)	25	13	9	3	-	-	-	0,700	0,300	-	[157]
		14	-	-	-	8	3	0,720	-	0,280	
Southeastern Europe											
Bosnian (Sarajevo, Eastern Bosnia)	129	96	32	1	-	-	-	0.868	0.132	-	[60]
		107	-	-	-	21	1	0.911	-	0.089	
Bosnian (Tešanj, Northern Central Bosnia)	43	35	8	0	-	-	-	0.910	0.090	-	[158]
Greek (Alexandroupolis, Northeastern Greece)	283	176	57	4	8	38	0	0,790	0,129	0,081	[159]
Kosovar (General population, Kosovo)	234	117	62	8	4	39	4	0,726	0,175	0,109	[160].
Macedonian (General population, Republic of North Macedonia)	179	112	38	5	2	20	2	0,788	0,139	0,073	[161]
Macedonian (Skopje, Northern Republic of North Macedonia)	27	18	5	0	0	4	0	0,833	0,093	0,074	[162]
Romanian (Cluj-Napoca, Northwestern Romania)	332	209	62	2	9	47	3	0,794	0,113	0,093	[104]
Serbian (General population, Serbia)	484	375	104	5	-	-	-	0,883	0,117	-	[163]
		411	-	-	-	68	5	0,919	-	0,081	
Turkey (Gaziantep, Southern Turkey)	499	308	90	5	6	86	4	0,794	0,106	0,100	[164]

Table 3.4 - Genotypic and allelic frequencies for the *CYP2C9**2 and *CYP2C9**3 polymorphisms among the European countries.(cont.).											
Population	N	Genotypic Distribution						Allelic Frequency			References
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
Turkish (Ankara, Norhern Central Turkey)	15	10	4	0	-	1	-	0,833	0,133	0,033	[165]
Turkish (Ankara, Norhern Central Turkey)	85	58	10	3	1	12	1	0,812	0,100	0,088	[166]
Turkish (Ankara, Norhern Central Turkey)	73	51	10	1	1	9	1	0,829	0,089	0,082	[167]
Turkish (Istanbul, Northwestern Turkey)	160	127	31	2	-	-	-	0,890	0,110	-	[168]
	142	114	-	-	-	18	10	0,870	-	0,130	
Turkish (Istanbul, Northwestern Turkey)	207	113	34	-	6	50	4	0,749	0,097	0,155	[106]
Turkish (Istanbul, Northwestern Turkey)	55	43	12	0	-	-	-	0,890	0,110	-	[169]
		48	-	-	-	6	1	0,940	-	0,060	
Turkish (Mersin, Southern Turkey)	186	119	35	3	8	20	1	0,788	0,132	0,081	[170]
Turkish (Mersin, Southern Turkey)	97	90	7	0	-	-	-	0,964	0,036	-	[171]
		77	-	-	-	20	0	0,897	-	0,103	
Turkish (Mersin, Southern Turkey)	90	74	14	2	-	-	-	0,900	0,100	-	[172]
		59	-	-	-	31	0	0,828	-	0,172	
Western Europe											
Belgian (Brussels, Northern Central Belgium)	120	81	22	-	2	14	1	0.825	0.100	0.075	[126]
British (Newcastle upon Tyne, Northeasthern England)	100	60	20	0	2	17	1	0,790	0,125	0,085	[173]
British (Newcastle upon Tyne, Northeasthern England)	128	81	23	3	3	16	2	0,785	0,125	0,090	[174]
British (General population, United Kingdom)	247	211	60	3	-	-	-	0,883	0,117	-	[175]
	280	233	-	-	-	47	0	0,916	-	0,084	
British (Unspecified city/region, United Kingdom)	76	69	-	-	-	5	2	0,941	-	0,059	[133]
British (Orkney Islands archipelago, Scotland)	88	77	-	-	-	11	0	0,937	-	0,063	
Population	N	*1/*1			*1/*2, *1/*3, *2/*2, *2/*3, *3/*3			*1	*2	*3	References
British (Tayside and Fife, East Central Scotland)	248	180			104			0,810	0,120	0,070	[124]
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
French (Paris, Northern Central France)	151	94	30	16	1	4	6	0,770	0,150	0,080	[176]

Table 5 - Genotypic and allelic frequencies for the *CYP2C9**2 and *CYP2C9**3 polymorphisms among the European countries (cont.).											
Population	N	Genotypic Distribution						Allelic Frequency			References
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
French (Paris, Northern Central France)	24	11	0	0	2	11	0	0,688	0,042	0,271	[120]
French (Paris, Northern Central France)	18	10	3	0	0	4	1	0,75	0,083	0,167	[177]
French (Paris, Northern Central France)	49	37	12	0	-	-	-	0,878	0,122	-	[178]
		42	-	-	-	7	0	0,929	-	0,071	
French (French Basque Country, Southwestern France)	76	64	-	-	-	11	1	0,914	-	0,086	[133]
Dutch (Amsterdam, Northwestern Netherlands)	93	75	-	-	-	18	0	0.905	-	0.095	[179]
		84	9	0	-	-	-	0.950	0.05	-	
Southern Europe											
Italian (General population, Italy)	174	101	36	6	11	16	4	0.730	0.176	0.095	[180]
Italian (Rome, Central Italy, and Sicily island)	90	76	11	2	0	1	0	0.910	0.083	0.007	[181]
Italian (Sicily Island, Italy)	360	223	62	10	8	52	5	0.778	0.125	0.097	[182]
Italian (Sicily Island, Italy)	59	51	-	-	-	8	0	0.932	-	0.068	[133]
Italian (Bergamo, Northeastern Italy)	81	75	-	-	-	6	0	0.963	-	0.037	
Italian (Piedmonte, Northern Italy)	58	51	-	-	-	6	1	0.931	-	0.069	
Italian (Verona, Nothern Italy) and Spanish (Santiago de Compostela, Northwestern Spain and Barcelona, Northeastern Spain)	1245	742	295	31	27	142	8	0.771	0.154	0.074	[183]
Italian (Sardinia Island, Italy)	384	245	89	9	8	32	1	0.797	0.15	0.056	[184]
Population	N	*1/*1		*1/*2, *1/*3		*2/*2,*2/*3, *3/*3		*1	*2	*3	References
Italian (Rome, Central Italy)	218	138		67		13		0.787	0.133	0.080	[185]
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
Portuguese (São Miguel island, Azores archipelago, Portugal)	58	35	9	2	5	7	0	0.741	0.155	0.103	[117]
Spanish (Madrid, Central Spain)	197	102	32	5	16	41	1	0.703	0.147	0.150	[186]
Spanish (Madrid, Central Spain)	24	13	4	2	-	5	-	0.729	0.167	0.104	[187]
Spanish (Madrid, Central Spain)	246	140	57	6	5	37	1	0.760	0.150	0.089	[188]

Table 5 - Genotypic and allelic frequencies for the *CYP2C9**2 and *CYP2C9**3 polymorphisms among the European countries (cont.).											
Population	N	Genotypic Distribution						Allelic Frequency			References
		*1/*1	*1/*2	*2/*2	*2/*3	*1/*3	*3/*3	*1	*2	*3	
Spanish (Madrid, Central Spain)	79	47	21	-	3	8	-	0.778	0.152	0.070	[189]
Spanish (Spanish Pyrenees region, Northeastern Spain)	154	96	29	4	6	18	1	0.769	0.142	0.088	[190]
Spanish (Badajoz, Southwestern Spain)	157	78	25	3	14	37	0	0.694	0.143	0.162	[191]
Spanish (Badajoz, Southwestern Spain)	102	59	20	4	4	14	1	0.745	0.156	0.098	[192]
Spanish (Badajoz, Southwestern Spain)	327	195	78	8	13	30	3	0.760	0.160	0.080	[193]
Spanish (Badajoz, Southwestern Spain)	362	237	11	14	-	-	-	0.808	0.192	-	[194]
		302	-	-	-	58	2	0.914	-	0.086	
Spanish (Badajoz, Southwestern Spain)	355	175	100	11	10	55	4	0.711	0.186	0.103	[195]
Spanish (Romani origin, Spain)	62	54	-	-	-	8	0	0.935	-	0.065	[133]
Spanish (Catalonia, Spain)	63	55	-	-	-	8	0	0.937	-	0.063	
Spanish (Valencia, Eastern Spain)	549	492	-	-	-	54	3	0.945	-	0.055	[196]
Spanish (Madrid, Central Spain and Alicante, Southeastern Spain)	26	16	4	-	-	5	1	0.788	0.077	0.135	[197]
Population	N	*1/*1		*1/*2, *1/*3		*2/*2,*2/*3, *3/*3		*1	*2	*3	References
Spanish (Zagaroza, Northeastern Spain)	282	164		104		14		0.790	0.133	0.077	[198]
		*1/*1		*1/*2, *1/*3		*2/*2,*2/*3, *3/*3		*1	*2	*3	
Spanish (Madrid, Central Spain)	106	66		37		3		0.800	0.120	0.070	[199]

Between Eastern European countries (Figure 3.1), only Russian individuals presented information regarding the *CYP2C9**2 and *CYP2C9**3 polymorphisms. Eight papers published between 2003 and 2020 surveyed 2438 control individuals' genotypic distribution belonging to twelve distinct ethnic groups (Balkar, Tatar, Russian, Bashkir, Avar, Lak, Dargin, Sámi, Chuvash, Mari, Kabardian, and Ossetian). These assessed volunteers were from ten different cities or republics in European Russia.

Among the Russian populations, several studied populations were separately assessed for the *CYP2C9**2 and *CYP2C9**3 polymorphisms. Within these studied populations are the Balkars and Tatars genotyped by Abdullaev et al. [127], the Russian (ethnic group), Tatars and Bashkirs studied by Korytina et al. [128], and the Chuvashs, Maris, Kabardin, and Ossetians studied by K. Mirzaev et al [88]. On the other hand, the Avars, Laks, and Dargins [129] as well as the Sámis [133] were only evaluated for the *CYP2C9**3 polymorphism's presence. Also, only the population from Moscow studied by O. Gra et al in 2008 [131] was not assessed for the *2/*2 genotype.

Among the Eastern European populations, only individuals belonging to the Russian ethnic group had their genotypic distribution fully assessed. These individuals were part of studied populations from Moscow [131] and Voronezh [132]. Only these populations can have their warfarin dose requirement predicted and compared.

The 352 individuals from Moscow [130] showed a *1/*2 (20.7%), *2/*2 (0.6%), *2/*3 (2.0%), *1/*3 (7.4%), and *3/*3 (0.3%) carriers' frequency that resulted, respectively in a *2 and *3 allele frequency of 11.9%, and 5.0%. Simultaneously, the 290 genotyped volunteers from Voronezh [132] presented a frequency for the 1/*2 (18.3%), *2/*2 (0.7%), *2/*3 (1.4%), *1/*3 (11.4%), and *3/*3 (0.3%) carriers that accounted for a *2 and *3 allele frequencies of 10.5%, and 6.7%, respectively. Such similar allelic frequencies are understandable since the genotyped individuals belong to the same ethnic group and Moscow and Voronezh are only 500km apart.

In Northern European populations (Figure 3.1, Table 3.4) twelve papers published between 1999 and 2012 assessed the presence of the *CYP2C9**2 and *CYP2C9**3 polymorphisms in 50833 healthy volunteers. The studied individuals were natives of Finland, Denmark, Sweden, and Norway.

The two samples of the Danish population [135], and the studied individuals from Turku in Finland [140] were separately assessed for the *CYP2C9**2 and *CYP2C9**3 polymorphisms. On the other hand, the population from Helsinki studied by Pimenoff et al. [133] only had the *CYP2C9**3 polymorphism's presence evaluated.

The remaining Northern European populations were fully genotyped. A total of 3067 studied individuals spread across study populations from the Faroe Islands [134], Odense [136], Copenhagen [137], Helsinki [138], [139], Oslo [134], and Stockholm [141]–[143] were assessed for the presence of the *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 genotypes.

The 276 studied individuals from Odense [136] did not register any carrier of the *3/*3 genotype and showed a *1/*2, *2/*2, *2/*3, and *1/*3 carriers' frequency, respectively of 19.2%, 1.4%, 2.2%, and 8.3%. The showed genotypic distribution for the studied individuals from Odense resulted in a *2 and *3 allele frequencies, respectively of 12.1%, and 5.3%. Similarly, the 175 genotyped controls from Copenhagen [137] showed a frequency for the *1/*2 (22.3%), *2/*2 (2.3%), *2/*3 (1.7%), *1/*3 (6.3%), and *3/*3 (0.6%) carriers that resulted in a *2 and *3 allele frequency, respectively of 14.3%, and 4.6%. These similar allelic frequencies are understandable since Odense, and Copenhagen are about 200 km apart.

Conversely, the 311 studied volunteers from the Faroe Islands [134] presented a frequency for the *1/*2 , *2/*3 , *1/*3 carriers, respectively of 16.1%, 1.7%, and 6.3%, and did not register any carrier for the *2/*2, and *3/*3 genotypes. This resulted in a *2, and *3 allele frequency of 8.8%, and 5.3%, respectively. This *2 allele frequency presented by the studied individuals from Faroe Islands differs from that presented by the studied populations from Copenhagen [137] (14.3%) and Odense [136] (12.1%). The reason for this to happen may lie on the remote location of the Faroe Islands in relation to continental Denmark.

The 12 individuals from Helsinki studied by Karonen et al. [139] did not register any carrier for the *2/*3, and *3/*3 genotypes, accounting for a *1/*2, *2/*2, and *1/*3 carriers' frequency of 8.3%. Also, the 29 volunteers from Helsinki studied by Niemi et al. [138] did not register a carrier for the *2/*3, and *3/*3 genotypes, showing a frequency for the *1/*2, *2/*2, and *1/*3 carriers respectively of 27.6%, 3.4%, and 10.3%. The populations from Helsinki studied by Karonen et al. [139], and Niemi et al. [138] showed disparate *2 (8.3%, and 15.5%, respectively) and *3 (8.3%, and 6.9%, respectively) allele frequencies. These disparate allelic frequencies are due to the small sample size presented by both studies. Thus, both studied populations will not be considered in further analysis with others for not being representative of the population of Helsinki.

The 430 volunteers from Stockholm studied by Yasar et al. in 1999 [142] presented a frequency for the *1/*2 (18.6%), *2/*2 (0.5%), *2/*3 (1.9%), *1/*3 (11.6%) and *3/*3 (0.7%) carriers, which resulted in a *2 and *3 allele frequency, respectively of 10.7%, and 7.4%. Similarly, 1503 controls also from Stockholm studied by Bennet et al. [143] showed a *1/*2 (17.9%), *2/*2 (1.4%), *2/*3 (1.2%), *1/*3 (11.0%) and *3/*3 (0.3%) carriers' frequency, that accounted for a *2 and *3 allele frequency, respectively of 11.0%, and 6.5%.

Conversely, the 22 individuals studied by Yasar et al. in 2002 [141] showed a frequency for the *1/*2 (13.6%), *2/*2 (13.6%), *2/*3 (18.2%), *1/*3 (22.7%) and *3/*3 (4.5%) carriers that resulted in a *2, and *3 allele frequency of 29.5%, and 25.0%, respectively. The allelic frequencies presented by this studied population studied by Yasar et al. in 2002 [141] and the remaining populations from Stockholm [142], [143] are disparate. This suggests that the population studied by Yasar et al. in 2002 [141] does not represent the population of Stockholm due to its small sample size. Hereupon, the individuals studied by Yasar et al. in 2002 [141] will not be considered in further analysis and comparisons.

The 309 studied individuals from Oslo in Norway [134], showed a *1/*2 (17.8%), *2/*2 (0.6%), *2/*3 (0.6%), *1/*3 (11.7%) and *3/*3 (0.3%) carriers' frequency that resulted in a *2 and *3 allele frequency, respectively of 9.9%, and 6.5%. The studied Norwegian presented a very similar *2 and *3 allele frequency with the Swedish studied by Yasar et al. in 1999 [142] (10.7%, and 7.4%, respectively), and Bennet et al. [143] (11.0%, and 6.5% respectively).

Amongst the studied Northern European populations, it is expectable that one Danish individual from the Faroe Islands is more likely to require a higher warfarin dose. This happens because the studied individuals from the Faroe Islands [134] present a lower *2 and *3 allele frequency, respectively with 8.8%, and 5.3%. On the other hand, one individual Stockholm is more prone to present a lower warfarin dose. This occurs due to the higher *2 and *3 allele frequencies presented by the Swedish genotyped in the two studies conducted by Yasar et al. in 1999 [142] (10.7%, and 7.4%, respectively) and Bennet et al. [143] (11.0%, and 6.5% respectively) [79].

The genotypic and allelic information found for the *CYP2C9**2 and *CYP2C9**3 polymorphisms in the Central European countries (Figure 3.1, Table 3.4) is available in 17 different papers. These studies involved 4491 healthy individuals from eight distinct countries, namely Austria, Croatia, Czech Republic, Germany, Hungary, Poland, Slovakia, and Switzerland. The assessed volunteers subjected to this evaluation were part of 15 study populations.

The German general studied population [149] and the studied individuals from Bonn [95] (Germany), and Łódź [154] (Poland), and Bern [157] (Switzerland) were separately assessed for the *CYP2C9**2 and *CYP2C9**3 polymorphisms. Moreover, the Croatians from Zagreb studied by Miroševic Skvrce et al. [147] did not have the *2/*3, and *1/*3 genotyped assessed. Also, another population from Zagreb studied by Božina et al. [125] was not evaluated for the presence of the *2/*3 genotype. Finally, a studied population from Poznań in Poland [155] was not assessed for the presence of the *2/*2, *2/*3, and *3/*3 genotypes.

On the other hand, in the remaining Central European populations, a total of 3305 individuals were assessed for the *1/*1, *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 genotypes. These volunteers were spread across study populations from Vienna [144] (Austria), Prague [148] (Czech Republic), Berlin [150] (Germany), Münster [151] (Germany), Würzburg [152] (Germany), Warsaw [97] (Poland), and Bratislava [156] (Slovakia). Also, one Hungarian [153], and two Croatian [145], [146] general study populations had their genotypic profile regarding the *CYP2C9**2 and *CYP2C9**3 completely assessed.

A group of 177 Croatians studied by Topić et al. [145] showed a *1/*2, *2/*2, *2/*3, and *1/*3 carriers' frequency, respectively of 23.2%, 6.8%, 0.6%, and 0.6%, and did not register any carrier for the *3/*3 genotype. Therefore, this studied population from Croatia accounted for a *2 and *3 allele frequency of 12.4%, and 3.7%, respectively. Conversely, the 1080 studied Croatians by Ganoci et al. [146] displayed a frequency for the *1/*2 (23.5%), *2/*2 (1.8%), *2/*3 (1.9%), *1/*3 (12.8%) and *3/*3 (0.3%) carriers that resulted in a *2 and *3 allele frequency of 14.5%, and 7.6%, respectively. The disparate allelic frequencies presented by these general study populations suggests that one of them is not representative of the Croatian population. Therefore, the population sample studied by Topić et al. [145] will be excluded from further analysis for being considerably lower than the sample studied by Ganoci et al. [146].

In Germany, three studied populations from Münster, Würzburg and Berlin were fully genotyped for the *CYP2C9**2, and *CYP2C9**3 polymorphisms. The 309 studied volunteers from Berlin [150] showed a *1/*2 (21.4%), *2/*2 (1.6%), *2/*3 (1.9%), *1/*3 (7.8%) and *3/*3 (1.0%) carriers' frequency that resulted in a *2 and *3 allele, respectively of 13.3%, and 5.8%. Similarly, the 283 studied individuals from Würzburg [152] presented a *1/*2 (18.4%), *2/*2 (1.8%), *2/*3 (1.8%), *1/*3 (13.8%) and *3/*3 (0.4%) carriers' frequency that accounted for a *2 and *3 allele frequency of 11.8%, and 8.1%, respectively. Also, the 66 genotyped volunteers from Münster [151] displayed a frequency for the *1/*2, *2/*2, *2/*3, *1/*3, respectively of 12.1%, 1.5%, 6.1%, and 15.2% and did not register any carrier of the *3/*3 genotype. Such genotypic distribution resulted in a *2 and *3 allele frequency, respectively of 14.0%, and 6.4%.

It is possible to verify that the three studied populations from Germany showed comparable allelic frequencies. However, the studied population from Münster [151] showed a disparate genotypic distribution with the studied populations from Würzburg [152], and Berlin [150], which is not expected since these are populations from cities of the same country. This suggests that the population sample from Münster [151] is too small to be representative. Therefore, the studied population from Münster will not be considered in further analysis.

The 375 studied Austrians from Vienna [144] showed a *1/*2, *2/*2, *2/*3, *1/*3, *3/*3 carriers' frequency, respectively of 21.1%, 1.9%, 0.8%, 10.7%, and 0.5%. Similarly, the 254 genotyped Czechs from Prague [148] presented a frequency for the carriers of the same genotypes, of 20.1%, 2.0%, 0.4%, 9.8%, and 0.8%, respectively. Moreover, the 535 studied Hungarians [153] presented a frequency for the same genotypes carriers' of 19.4%, 2.1%, 1.5%, 13.8%, and 1.1%, respectively. Also, the 112 genotyped Slovaks [156] from Bratislava did not register any carrier of the *3/*3 genotype and accounted for a *1/*2, *2/*2, *2/*3, *1/*3 carriers' frequency, respectively of 14.3%, 1.8%, 2.7%, and 12.5%. Such similar genotypic distributions for the studied Austrians, Czechs, Hungarians, and Slovaks resulted in an also similar *2 (12.8%, 12.2%, 12.5%, and 11.9% respectively), and *3 (6.3%, 5.9%, 8.8%, and 5.0% respectively) allele frequency. This can be explained due to geographical proximity of these countries.

On the other hand the 114 studied Polish [97] showed a disparate *3 allele frequency with the studied Austrians [144], Czechs [148], Hungarians [153], and Slovaks [156] despite being neighboring populations. The studied Polish presented a *1/*2, *2/*3, and *1/*3 carriers' frequency, respectively of 14.9%, 4.4% and 16.7% and did not register any carrier of the *2/*2, and *3/*3 genotypes. This genotypic distribution resulted in a *2 and *3 allele frequency, respectively of 9.6%, and 10.5%.

Amongst the Central European populations, one Czech individual is more prone to require a higher warfarin dose. This is due to the lower *2 and *3 allele frequency presented by the studied population from Czech Republic [148], respectively with 12.2% and 5.9%. Conversely, one Polish individual is expected to require a lower warfarin dose requirement. The reason for this to happen is the result of the higher *2, and *3 allele frequencies presented by the studied population from Poland [97], respectively with 9.6%, and 10.5% [79].

The information concerning the presence of the *CYP2C9**2 and *CYP2C9**3 polymorphisms in the Southeastern European countries is available in 18 papers published between 2004 and 2019. Study populations from Bosnia and Herzegovina, Greece, Kosovo, Macedonia, Romania, Serbia, and Turkey (Figure 3.1, Table 3.4) involved a total of 3178 healthy volunteers dispersed among 12 different populations.

The Serbian general population sample [163] and the studied individuals from Sarajevo [60] (Bosnia and Herzegovina), Istanbul [168], [169] (Turkey), and Mersin [171], [172] (Turkey) were separately assessed for the *CYP2C9**2 and *CYP2C9**3 polymorphisms. Moreover, the Bosnian studied population from Tešanj [158] only had the *CYP2C9**3 polymorphism's presence assessed. Also, the studied individuals from Ankara [165] (Turkey) were not assessed for the *2/*3, and *3/*3 genotypes. Finally, the Istanbul population studied by Taşkın [106] did not have the *2/*2 genotype evaluated.

Conversely, 1898 healthy individuals were fully genotyped for the presence of the *CYP2C9**2, and *CYP2C9**3 polymorphisms in the remaining Southeastern populations. These volunteers were spread across populations from Alexandroupolis [159] (Greece), Skopje [162] (Macedonia), Cluj-Napoca [104] (Romania), Gaziantep [164] (Turkey), Mersin [170] (Turkey), and Ankara [166], [167] (Turkey). Also, two general study populations from Kosovo [160], and Macedonia [161] were fully genotyped.

The 27 studied individuals from Skopje [162] showed a frequency for the *1/*2, and *1/*3 carriers of respectively, 18.5%, and 14.8% and did not register any carrier for the *2/*2, *2/*3, and *3/*3 genotypes. This genotypic distribution accounted for a *2 and *3 allele frequency, respectively of 9.4%, and 7.4%. On the other hand, the 179 studied individuals part of Macedonian population sample [161] presented a frequency for the same genotype's carriers genotypes of 21.2%, 2.8%, 1.1%, 11.2%, and 1.1%, respectively. This studied population's *2 and *3 allele frequency then accounted, respectively for 13.9%, and 7.3%. The presented disparities between the two Macedonian studied populations occur due to the small sample size of the population studied by Kapedanovska Nestorovska et al. [162]. Therefore, the genotyped population from Skopje is not representative, and for that reason will not be included in further analysis.

The 283 studied Greeks [159] showed a *1/*2, *2/*2, *2/*3, and *1/*3 carriers' frequency, respectively of 20.1%, 1.4%, 2.8%, and 13.4% and did not register any carrier of the *3/*3 genotype. Similarly, the 332 genotyped Romanians [104] showed a *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers' frequency, respectively of 18.7%, 0.6%, 2.7%, 14.2% and 0.9%. The Romanian, Greek, and Macedonian [161] studied populations showed, as expected, a similar *2 (11.3%, 12.9%, 13.9%, respectively), and *3 (9.3%, 8.1%, 7.3%, respectively) allele frequency. Such comparable frequencies occur because these are populations from neighboring countries. Conversely, the 234 studied Kosovars [160] presented a *1/*2 (26.5%), *2/*2 (3.4%), *2/*3 (1.7%), *1/*3 (16.7%), and *3/*3 (1.7%) carriers' frequency that accounted for the highest *2 and *3 allele frequency, respectively with 17.5%, and 10.9%.

In Turkish studied populations a total of 843 healthy individuals from Ankara, Gaziantep, and Mersin were fully genotyped. The 85 individuals from Ankara studied by Babaoglu et al. [166] showed a frequency for the *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers, respectively of 11.8%, 3.5%, 1.2%, 14.1%, 1.2%. Similarly the 73 individuals also from Ankara studied by Goktaş et al. [167] showed a comparable frequency for the sames genotypes' carriers of 13.7%, 1.4%, 1.4%, 12.3%, and 1.4%, respectively. These shown genotypic distributions for populations studied by Babaoglu et al. [166], and Goktaş et al. [167] resulted in a comparable *2 (10.0%, and 8.8%, respectively) and *3 (8.9%, and 8.2%, respectively) allele frequencies.

Also, the 186 studied individuals from Mersin [170] and the 499 genotyped volunteers from Gaziantep [164] showed similar *2 and *3 allele frequencies. The studied population from Mersin presented a *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers' frequency, respectively of 18.8%, 1.6%, 4.3%, 10.8%, and 0.5%. Regarding the genotyped population from Gaziantep they showed a frequency for the same genotypes' carriers of 18.0%, 1.0%, 1.2%, 17.2%, and 0.8%, respectively. Therefore, the genotypic distributions presented by the studied populations from Mersin and Gaziantep resulted in comparable *2 (13.2%, and 10.6%, respectively) and *3 (8.1%, and 10.0%, respectively) allele frequencies. Such similar allelic frequencies are understandable since the cities of Mersin and Gaziantep are only 300 km apart.

It is possible to verify a similar *2 and *3 allele frequencies amongst the Southeastern European populations, excluding the case of the Kosovar studied population. For this reason, one individual from Kosovo is expected to require a lower warfarin dose. This is the result of the higher *2 and *3 allele frequencies presented by the studied population from Kosovo, respectively with 17.5%, and 10.9%. On the other hand, the one individual from Ankara is more prone to require a higher warfarin dose. The explanation for this lie on the lower *2 and *3 allele frequencies showed by the populations studied by Babaoglu et al. (10.0%, and 8.8%, respectively) [166], and Goktaş et al. (8.9%, and 8.2%, respectively) [167] [79].

The 13 studies conducted in Western European populations (Figure 3.1, Table 3.4) assessed 1451 healthy individuals for the presence of the *CYP2C9**2 and *CYP2C9**3 polymorphisms. These studied individuals were spread across nine populations from Belgium, France, Netherlands, and the United Kingdom (UK). The papers with the mentioned information were published between 1999 and 2017.

The British general population sample [175], the studied individuals from Paris [178] (France), and Amsterdam [179] (Netherlands) were separately genotyped for the *CYP2C9**2, and *CYP2C9**3 polymorphisms. Also, the studied individuals from Orkney Islands [133] (UK), French Basque Country [133] (France), and the British from an unspecified region [133] were only assessed for the presence of the *CYP2C9**3 polymorphism. Moreover, one studied population from Brussels [126] (Belgium) was not assessed for the *2/*2 genotype.

At the same time, 421 individuals had the presence of the *CYP2C9**2, and *CYP2C9**3 polymorphisms completely assessed. These volunteers were spread across studied populations from Newcastle [173], [174] (UK), Tayside and Fife [124] (UK) and Paris [120], [176], [177] (France).

Two populations from Newcastle upon Tyne were fully genotyped by Aithal et al. [173], and Howard et al. [174], involving a total of 228 volunteers. The 100 individuals studied by Aithal et al. [173] did not register any carrier for the *2/*2 genotype, and showed a *1/*2, *2/*3, *1/*3, and *3/*3 carriers' frequency respectively of 20.0%, 2.0%, 17.0%, and 1.0%. Similarly, the 128 volunteers studied by Howard et al. [174] presented a frequency for the same genotypes' carriers of 18.0%, 2.3%, 2.3%, 12.5%, 1.6%, respectively. The *2 and *3 allele frequencies are, as expected very similar for the populations studied by Aithal et al. [173] (12.5%, and 9.0%, respectively), and Howard et al. [174] (12.5%, and 8.5%, respectively).

Also, the 248 studied individuals from Tayside and Fife [124] despite not presenting the discriminate number of the carriers for each genotype, were fully genotyped. This studied population presented such a genotypic distribution that resulted in a similar *2 (12.0%) and *3 (7.0%) allele frequency with that presented by the two studied populations from Newcastle upon Tyne [173], [174]. The similar allelic frequencies can be explained due to the geographical proximity since the city of Newcastle upon Tyne and the region of Tayside and Fife are only 250 km apart.

In France, three populations from Paris were genotyped, involving a total of 193 volunteers. The group of 24 individuals studied by Verstuyft et al. [120] did not register any carrier for the *1/*2, *2/*2, and *3/*3 genotypes, presenting a *2/*3, and *1/*3 carriers' frequency, respectively of 8.3%, and 45.8%. Another group of 18 individuals studied by Chhun et al. [177], they did not register any carrier of the *2/*2, and *2/*3 genotypes accounting for a *1/*2, *1/*3, and *3/*3 carriers' frequency, respectively of 16.7%, 22.2%, and 5.6%. On the other hand, the 151 volunteers studied by J. Q. Yang et al. [176] presented a *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers' frequency, respectively of 19.9%, 2.6%, 4.0%, 10.6%, and 0.7%. The *2 and *3 allele frequencies then differed between the populations

studied by Verstuyft et al. [120] (4.2%, and 27.1%, respectively), Chhun et al. [177] (8.3%, and 16.7%, respectively), and J. Q. Yang et al. [176] (15.0%, and 8.0%, respectively). The disparate allelic frequencies presented can be explained by the small sample size of the populations studied by Verstuyft et al. [120], and Chhun et al. [177]. Therefore, the populations studied by Verstuyft et al. [120], and Chhun et al. [177] will be excluded from further analysis for not being representative of the population of Paris.

Amongst the Western European studied populations one individual from the region of Tayside and Fife is more likely to require a higher warfarin. This happens because the studied population from Tayside and Fife presented a lower *2, and *3 allelle frequencies, respectively with 12.0%, and 7.0%. On the other hand, one individual from Paris is more prone to require a lower warfarin dose. The reason for this is because of the higher *2 and *3 allele frequencies presented by the population from Paris studied by J. Q. Yang et al. [176], respectively with 15.0%, and 8.0% [79].

The presence of the *CYP2C9**2, and *CYP2C9**3 polymorphisms in Southern European countries (Figure 3.1, Table 3.4) is available in 22 papers published between 1999 and 2020. These papers involved 6160 healthy individuals spread across 17 populations from Portugal, Spain, and Italy.

Only one population from Badajoz studied by Martínez et al. in 2014 [194] (Spain) had the presence of the *CYP2C9**2, and *CYP2C9**3 polymorphisms separately assessed. Also, studied populations from Valencia [196] (Spain), Sicily [133] (Italy), Bergamo [133] (Italy), Piedmonte [133] (Italy), Catalonia [133] (Spain), and the genotyped individuals from a Romani community [133] (Spain) were only assessed for the presence of the *CYP2C9**3 polymorphism. Moreover, one population from Madrid studied by Cabaleiro et al. in 2015 [189] was not assessed for the *2/*2, and *3/*3 genotypes. Furthermore, one studied population grouping individuals from Madrid and Alicante [197] did not have the *2/*2, and *3/*3 genotypes evaluated. Additionally, one population from Madrid studied by Dapia et al. [199] did not have the *3/*3 genotype assessed. Finally, one population from Madrid studied by Zubiaur et al. [187] was not assessed for the*2/*3, and *3/*3 genotypes.

Within the remaining Southern European populations there are the 4349 fully genotyped individuals for the presence of the *CYP2C9**, and *CYP2C9**3 polymorphisms. The volunteers were part of study populations from Rome [185] (Italy), Sicily [182] (Italy), Sardinia [184] (Italy), São Miguel [117] (Portugal), Madrid [186], [188] (Spain), Badajoz [191]–[193], [195] (Spain), Zaragoza [198] (Spain) and the Spanish Pyrenees region [190]. Also, one Italian general population sample [180], one grouped population involving volunteers from Verona (Italy), Santiago de Compostela (Spain), and Barcelona (Spain) [183], and other involving controls from Rome and Sicily [181] were fully genotyped.

The 218 studied volunteers from Rome [185] do not present the discriminated numbers for the carriers of each genotype. Despite this, this population was completely genotyped and showed a *2, and *3 allele frequency, respectively of 13.3%, and 8.0%, respectively. Similarly, the 360 genotyped islanders from Sicily [182] presented a *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers' frequency, respectively of 17.2%, 2.8%, 2.2%, 14.4%, and 1.4%. The resulting *2 and *3 allele frequencies presented for the studied individuals from Sicily (12.5%, and 9.7%, respectively) are then comparable to that presented by the genotyped population from Rome. Although Sicily is an island, its populations is not very isolated from continental Italy, which may explain the similarities on the allelic frequencies.

The study involving 90 studied volunteers from Rome and Sicily [181] do not discriminate the number of individuals from each city. For that reason, the shown data for this sample refers to Rome and Sicily population. The presented *1/*2, *2/*2, *1/*3 carriers' frequency was, respectively, 12.2%, 2.2%, and 1.1%, while the *2/*3, and *3/*3 genotypes did not register any carrier. This genotypic distribution resulted in an *2 and *3 allele frequency, respectively of 8.3%, and 0.7%, which differs from that presented by the studied populations from Rome [185] (13.3%, and 8.0%, respectively) and Sicily [182] (12.5%, and 9.7%, respectively). Therefore, it is possible to verify that the studied population sample from Rome and Sicily is not representative, and for that reason, will be excluded from further analysis.

A group of 218 studied individuals belonging to the Italian general population sample [180] were from northern, central, and southern continental Italy. These individuals showed a *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers' frequency, respectively of 20.7%, 3.4%, 6.3%, 9.2%, and 2.3%. This genotypic distribution resulted in a *2 and *3 allele frequency, respectively of 17.6%, and 9.5%. The *2 and *3 allele frequencies presented by the studied individuals from Rome [185] (13.3%, and 8.0%, respectively) and Sicily [182] (12.5%, and 9.7%, respectively) does not differ from those showed by the Italian general population sample.

The 384 studied volunteers from Sardinia [184] displayed a frequency for the *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers, respectively of 23.2%, 2.3%, 2.1%.8.3%, and 0.3%. This genotypic distribution resulted in a *2 and *3 allele frequencies, respectively of 15.4%, and 5.6%. This presented *2 and *3 allele frequencies contrast with that presented by the studied individuals from [185] (13.3%, and 8.0%, respectively), Sicily [182], and the Italians part of the general population sample sample [180] (17.6%, and 9.5%, respectively). The reason for this is due to the isolation of the island of Sardinia, which allows its population to preserve its unique genetic code since the Neolithic period [200].

A total of 941 studied individuals from Badajoz spread across four studied populations [191]–[193], [195] had the presence of the *CYP2C9**2 and CYP2C9*3 assessed. A group of 355 genotpyped individuals studied by Martínez et al. in 2005 [195] presented a *1/*2 (28.2%), *2/*2 (3.1%), *2/*3 (2.8%), *1/*3 (15.5%), and *3/*3 (1.1%) carriers' frequency that resulted in a *2, and *3 allele frequency, respectively of 18.6%, and 10.3%. Simultaneously, another group of 327 volunteers studied by Pedro Dorado et al. in 2011 [193] showed frequency for the same genotypes' carriers of 23.9%, 2.4%, 4.0%, 9.2%, and 0.9%, respectively. This genotypic distribution resulted in a *2 and *3 allele frequencies, respectively of 16.0%, and 8.0%.

Also, the 102 controls studied by Pedro Dorado et al. in 2003 [192] volunteers displayed a frequency for the *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers, respectively of 19.6%, 3.9%, 3.9%, 13.7%, and 1.0%. This accounted for a *2, and *3 allele frequencies, respectively of 15.6%, and 9.8% for the individuals studied by Pedro Dorado et al. in 2003 [192].

On the other hand, the 157 individuals studied by Elena García-Martín in 2001 [191] did not register any carrier for the *3/*3 genotype, and presented a *1/*2, *2/*2, *2/*3, and *1/*3 carriers' frequency, respectively of 15.9%, 1.9%, 8.9%, and 23.6%. This genotypic distribution accounted for a *2 and *3 allele frequencies, respectively of 14.3%, and 16.2%. These presented frequencies for the *2 and *3 allele differ from the remaining studied populations from Badajoz. Therefore, such disparate allelic frequencies suggest that the population studied by Elena García-Martín in 2001 [191] is not representative of the population of Badajoz and will be excluded from further analysis.

The studied populations from Zaragoza [198], and the Pyrenees region [190] showed similar frequencies for the *2 and *3 allele. The 282 studied individuals from Zaragoza had not discriminated

the number of carriers for each genotype. The 154 assessed controls from the Pyrenees region showed a *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers' frequency, respectively of 18.8%, 2.6%, 3.9%, 11.7%, and 0.6%. The *2 and *3 allele frequencies were then very similar among the studied populations from Zaragoza (13.3%, and 7.7%, respectively), and the Pyrenees region (14.0%, and 8.4%, respectively)

Also, the 246 individuals from Madrid studied by Cabaleiro et al. in 2012 [188] presented a frequency for the *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers, respectively of 23.2%, 2.4%, 2.0%, 15.0%, and 0.4%. This accounted for a *2 and *3 allele frequencies, respectively of 15.0%, and 8.9%, very similar with that presented by the studied population from Zaragoza [198] (13.3%, and 7.7%, respectively) which is only 300 km apart from Madrid.

A group of 197 volunteers studied by Elena García-Martín et al. in 2002 [186] presented a frequency for the *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers, respectively of 16.2%, 2.5%, 8.1%, 20.8%, and 0.5%. This resulted in a *2 and *3 allele frequencies, respectively of 14.7%, and 15.0% which are very disparate from that presented by the population from Madrid studied by Cabaleiro et al. in 2012 [188] (15.0%, and 8.9%, respectively). Therefore, this study population [186] will be excluded from further analysis for not being representative of the population from Madrid.

The 58 volunteers from São Miguel [117] did not register any carrier of the *3/*3 genotype, and presented a *1/*2, *2/*2, *2/*3, *1/*3 carriers' frequency, respectively of 15.5%, 3.4%, 8.6%, and 12.1%. Similarly, the grouped Italian-Spanish population sample [183] showed a frequency for the *1/*2, *2/*2, *2/*3, *1/*3, and *3/*3 carriers, respectively of 23.7%, 2.5%, 2.2%, 11.4%, and 0.6%. This resulted in similar *2 and *3 allele frequencies for the studied Portuguese (15.5%, and 10.3%, respectively), and the Italian-Spanish group sample (15.4%, and 7.4%, respectively). These assessed individuals also showed similar *2 and *3 allele frequencies with the studied populations from Madrid [188] (15.0%, and 8.9%, respectively), Zaragoza [198] (13.3%, and 7.7%, respectively), and Rome [185] (13.3%, and 8.0%, respectively).

Amongst the Southern European populations, one individual from Badajoz is more likely to require a lower warfarin dose. This is due to the higher *2 and *3 allele frequencies presented by the individuals from Badajoz studied by Pedro Dorado et al. in 2003 [192] (15.6%, and 9.8%, respectively), and 2011 [193] (16.0%, and 8.0%, respectively), as well as the controls studied by Martínez et al. in 2005 [195] (18.6%, 10.3%, respectively). On the other hand, one individual from the Sardinia island is more expectable to present a higher warfarin dose requirement. This occurs since the studied population from Sardinia showed a lower *2 and *3 allele frequencies, respectively of 15.0%, and 5.6% [79].

3.5. CYP4F2 V433M gene polymorphism frequency in European countries

In this work, five studies published between 2014, and 2020 involved a total of 1777 volunteers assessing the presence of the V433M polymorphism in seven European populations. These volunteers were spread across Hungary [201], Lithuania [202], Russia [127], Slovakia [98] and Spain [203]. The age and gender of the participants were not referred in the included studies. The genotypic distribution for the V433M polymorphism and its allelic frequency is available in Table *3.5*.

Table 3.5 – Genotypic and allelic frequencies for the CYP4F2 V433M gene polymorphism among the European countries.

Population	N	Genotype distribution			Allelic frequency		Reference
		GG	GA	AA	G	A	
Eastern Europe							
Russian Balkar (Kabardino-Balkarian Republic, Southwestern European Russia)	200	48	104	48	0.500	0.500	[127]
Russian Tatar (Republic of Tatarstan, Eastern European Russia)	141	91	41	9	0.791	0.209	[127]
Central Europe							
Romani (Pecs, Southwestern Hungary)	484	225	206	53	0.678	0.322	[201]
Lithuanian (Kaunas, Central Lithuania)	210	112	82	16	0.729	0.271	[202]
Hungarian (Pecs, Southwestern Hungary)	493	247	208	38	0.712	0.288	[201]
Slovak (Brastislava, Southwestern Slovakia)	112	53	55	4	0.719	0.281	[98]
Southern Europe							
Spanish (Badajoz, Southwestern Spain)	137	53	71	13	0.646	0.354	[203]

The 484 genotyped Romani individuals [201] displayed a frequency for the for the G/A, and A/A carriers, respectively of 42.6%, and 11.0%. Simultaneously, the 493 studied volunteers from Hungary [201] presented a G/A, and A/A carriers' frequency, respectively of 42.2% and 7.7%. Simultaneously, the 112 studied individuals from Slovakia [98] presented a G/A, and a A/A carriers' frequency, respectively of 49.1%, and 3.6%. Simultaneously, the 210 genotyped Lithuanians [202] accounted for a frequency of the G/A, and A/A carriers respectively of 39.0%, and 7.6%. This resulted in a similar A allele frequency amongst the Central European studied populations of 32.2%, 28.8%, 28.1%, and 27.1%, respectively for the genotyped Romani, Hungarians, Slovaks, and Lithuanians.

The 137 studied Spanish [203] presented a G/A, and A/A carriers' frequency, respectively of 51.8%, and 9.5%. Such genotypic distribution will then result in an A allele frequency of 35.4%. This resulting A allele frequency compares to that obtained by the studied Hungarians [201] (32.2%).

On the opposite side, the 200 studied Russian Balkars from the Republic of Kabardino-Balkarian presented a frequency for the G/A, and A/A carriers of 52.0%, and 24.0%, respectively. The 141 studied Russian Tatars showed a G/A, an A/A carriers' frequency, respectively of 29.1%, and 6.4%. This resulted in a disparate A allele frequency of 50.0%, and 20.9%, respectively for the genotyped Balkars, and Tatars. Such a disparate A allele frequencies occur because these are two studied populations belonging to different ethnic groups. [127].

As the data for the V443M presence is scarce, the only conclusions that can be drawn are based on the frequencies shown in Table 3.*5*. Among these studied populations, the studied Balkars showed the highest frequency for the A allele with 50.0%, while the Tatars studied showed the lowest with 20.9%. Hereupon, this means that one Tatar individual is more likely to require a higher warfarin dose and to be less susceptible to suffer from CVDs. On the other hand, one Balkar individual is expected to require a lower warfarin dose and to be more prone to suffer from CVDs [81], [82], [127].

CONCLUSIONS

In this dissertation, it was proposed to study compile data regarding frequency of *VKORC1*, CYP2C9 and *CYP4F2* gene polymorphisms in European populations and relate it to their required warfarin dose.

The studied populations from Central Europe, and adjacent countries showed a frequency for the -1639 G>A polymorphism (*VKORC1*) lower than 43.1%. Conversely, the populations from Eastern and Southeastern European countries where the Turkish presence is preponderant registered a frequency for the -1639 G>A polymorphism in a range of values from 44.4% to 53.4%. Therefore, one individual from Russia, Turkey and Cyprus is more likely to require a lower warfarin dose because their populations presented a higher frequency of the -1639 G>A polymorphism.

In the assessed populations from Central, Western, and Southern European countries the presence of the 1173 C>T (*VKORC1*) polymorphism was lower than 44.8%. On the other hand, the genotyped individuals from Southeastern European countries accounted for a higher frequency of the 1173 C>T polymorphism with a range of values from 46.7% to 50.0%. It is possible to verify that one individual from a Southeastern country is more prone to require a lower warfarin dose since their populations presented a higher presence of this polymorphism.

In the studied populations from continental Southern Europe, and neighboring countries the presence of the *CYP2C9**2 polymorphism accounted for a range of values from 12.5% to 18.6%. Also, the presence of the *CYP2C9**3 polymorphism varied from 7.3% to 10.9%. Conversely, the *CYP2C9**2 polymorphism presented a range of values from 8.8% to 14. 3% in studied populations from Northern, Eastern Europe, and adjacent countries. Regarding the presence of the *CYP2C9**3 polymorphism, it varies from 4.6% to 7.4%. Considering the higher presence these polymorphisms in the populations, one individual from Spain, Italy, and Kosovo is more prone to require a lower warfarin dose

Regarding the presence of the V433M (*CYP4F2*), and 3730 G>A (*VKORC1)* polymorphisms no conclusions can be drawn. This is due to the scarce information regarding their frequency across European countries.

Based on these data, it is also possible to verify that the presence of these polymorphisms in populations does not depend on borders, languages, or creeds. On the contrary, it is explained by migratory phenomena that may have been the result of wars, natural disasters, financial crises, among others. For this reason, the studied individuals from isolated communities or lands will tend to present more extreme values for the MAF.

The studied populations may show a higher frequency for a polymorphism of a particular gene, while showing a lower for another. This happens because the considered genes (*VKORC1*, *CYP2C9*, and *CYP4F2*) are located in different chromosomal regions. For this reason, it would not be expected that polymorphisms of different genes presented concordant frequencies in a particular population.

REFERENCES

[1] T. S. Huang *et al.*, 'DNA sensors to assess the effect of VKORC1 and CYP2C9 gene polymorphisms on warfarin dose requirement in Chinese patients with atrial fibrillation', *Australas. Phys. Eng. Sci. Med.*, vol. 40, no. 1, pp. 249–258, 2017.

[2] J. D. Horton and B. M. Bushwick, 'Warfarin therapy: Evolving strategies in anticoagulation', *Am. Fam. Physician*, vol. 59, no. 3, pp. 635–646, 1999.

[3] R. M. Turner, M. Bula, and M. Pirmohamed, *Personalized Medicine in Cardiovascular Disease*. Elsevier Inc., 2017.

[4] M. D. Caldwell *et al.*, 'CYP4F2 genetic variant alters required warfarin dose', vol. 111, no. 8, pp. 4106–4113, 2008.

[5] M. Nichols, N. Townsend, P. Scarborough, and M. Rayner, 'Estimating an individual person's course of coronary artery calcification', *Eur. Heart J.*, vol. 35, no. 42, p. 2931, 2014.

[6] M. Nichols, N. Townsend, and M. Rayner, 'European cardiovascular disease statistics', *Eur. Hear. Netw. Eur. Soc. Cardiol.*, pp. 10–123, 2012.

[7] E. Wilkins, L. Wilson, K. Wickramasinghe, and P. Bhatnagar, 'European Cardiovascular Disease Statistics 2017', *Eur. Hear. Netw.*, pp. 94–100, 2017.

[8] L. Ward, *Sex differences in atherosclerosis and exercise effects*, no. May. 2019.

[9] H. Thomas *et al.*, 'Global Atlas of Cardiovascular Disease 2000-2016: The Path to Prevention and Control', *Glob. Heart*, vol. 13, no. 3, pp. 143–163, 2018.

[10] B. V. Reamy, P. M. Williams, and D. P. Kuckel, 'Prevention of Cardiovascular Disease', *Prim. Care - Clin. Off. Pract.*, vol. 45, no. 1, pp. 25–44, 2018.

[11] World Health Organization, 'Prevention of Cardiovascular Disease: Pocket guidelines with cardiovascular risk prediction charts for assessment and management of cardiovascular risk', 2007. .

[12] K. S. Chandra, M. Bansal, M. T. Medicity, T. Nair, and S. S. Iyengar, 'Consensus statement on management of dyslipidemia in Indian subjects ScienceDirect Consensus statement on management of dyslipidemia in Indian subjects', no. December, 2014.

[13] A. M. Clark, M. DesMeules, W. Luo, A. S. Duncan, and A. Wielgosz, 'Socioeconomic status and cardiovascular disease: Risks and implications for care', *Nat. Rev. Cardiol.*, vol. 6, no. 11, pp. 712–722, 2009.

[14] T. A. Gaziano, A. Bitton, S. Anand, S. Abrahams-Gessel, and A. Murphy, 'Growing Epidemic of Coronary Heart Disease in Low- and Middle-Income Countries', *Curr. Probl. Cardiol.*, vol. 35, no. 2, pp. 72–115, 2010.

[15] Eurostat, 'Real GDP per capita', *Eurostat*, 2020. [Online]. Available: https://ec.europa.eu/eurostat/databrowser/view/sdg_08_10/CustomView_1/table?lang=en. [Accessed: 07-Sep-2020].

[16] Eurostat, 'Deaths from cardiovascular diseases 2016', 2016. .

[17] A. Habib, G. Petrucci, and B. Rocca, 'Pathophysiology of Thrombosis in Peripheral Artery Disease', *Pediatr. Res.*, vol. 86, no. 1, pp. 19–27, 2020.

[18] J. Knight, C. Caseldine, and M. T. Boykoff, 'Forum review', *Geogr. J.*, vol. 176, no. 3, pp. 267–269, 2010.

[19] S. A. Smith, R. J. Travers, and J. H. Morrissey, 'How it all starts: Initiation of the clotting cascade', *Crit. Rev. Biochem. Mol. Biol.*, vol. 50, no. 4, pp. 326–336, 2015.

[20] S. P. Grover and N. Mackman, ‘Intrinsic pathway of coagulation and thrombosis: Insights from animal models’, *Arterioscler. Thromb. Vasc. Biol.*, vol. 39, no. 3, pp. 331–338, 2019.

[21] S. Perez-Pujol, O. Aras, and G. Escolar, ‘Factor V Leiden and Inflammation’, *Thrombosis*, vol. 2012, pp. 1–10, 2012.

[22] K. Nogami and M. Shima, ‘New therapies using nonfactor products for patients with hemophilia and inhibitors’, *Blood*, vol. 133, no. 5, pp. 399–406, 2019.

[23] M. Wadelius *et al.*, ‘Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors’, *Pharmacogenomics J.*, vol. 4, no. 1, pp. 40–48, 2004.

[24] T. T. S. Flora Darcy R, Rettie Allan E, Brundage Richard C, ‘CYP2C9 Genotype-Dependent Warfarin Pharmacokinetics: Impact of CYP2C9 Genotype on R- and S-Warfarin and Their Oxidative Metabolites.’, *J. Clin. Pharmacol.*, vol. 53, no. 9, pp. 1689–1699, 2017.

[25] J. S. Teles, E. Y. Fukuda, and D. Feder, ‘Varfarina : perfil farmacológico e interações medicamentosas com antidepressivos Warfarin : pharmacological profile and drug interactions with antidepressants’, *Einstein*, vol. 10, no. 1, pp. 110–115, 2012.

[26] G. E. Delgado *et al.*, ‘The association of high-normal international-normalized-ratio (INR) with mortality in patients referred for coronary angiography’, *PLoS One*, vol. 14, no. 8, pp. 1–14, 2019.

[27] M. J. Heron, ‘What is a Therapeutic Community ?’, *Br. Med. J.*, vol. 1, no. 5429, p. 250, 1965.

[28] A. Palermo *et al.*, ‘Vitamin K and osteoporosis: Myth or reality?’, *Metabolism.*, vol. 70, pp. 57–71, 2017.

[29] X. Gong, R. Gutala, and A. K. Jaiswal, ‘Quinone Oxidoreductases and Vitamin K Metabolism’, *Vitam. Horm.*, vol. 78, no. 07, pp. 85–101, 2008.

[30] J. K. Tie and D. W. Stafford, *Functional Study of the Vitamin K Cycle Enzymes in Live Cells*, 1st ed., vol. 584. Elsevier Inc., 2017.

[31] C. G. Bevans *et al.*, ‘Determination of the warfarin inhibition constant Ki for vitamin K 2,3-epoxide reductase complex subunit-1 (VKORC1) using an in vitro DTT-driven assay’, *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1830, no. 8, pp. 4202–4210, 2013.

[32] J. Y. Chyou, J. L. Mega, and M. S. Sabatine, *Pharmacogenetics*, Fourth Edi. Elsevier Inc., 2013.

[33] S. Wu, X. Chen, D. Y. Jin, D. W. Stafford, L. G. Pedersen, and J. K. Tie, ‘Warfarin and Vitamin K epoxide reductase: a molecular accounting for observed inhibition’, *Blood*, vol. 132, no. 6, pp. 647–657, 2018.

[34] J. Ansell, J. Hirsh, L. Poller, H. Bussey, A. Jacobson, and E. Hijlek, ‘The pharmacology and management of the vitamin K antagonists: The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy’, *Chest*, vol. 126, no. 3 SUPPL., pp. 204S-233S, 2004.

[35] J. Ansell, J. Hirsh, E. Hylek, A. Jacobson, M. Crowther, and G. Palareti, ‘Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition)’, *Chest*, vol. 133, no. 6 SUPPL. 6, pp. 160S-198S, 2008.

[36] S. Wessler and S. Gitel, ‘Warfarin: From Bedside to Bench’, 1984.

[37] K. Harter, M. Levine, and S. O. Henderson, ‘Anticoagulation drug therapy: A review’, *West. J. Emerg. Med.*, vol. 16, no. 1, pp. 11–17, 2015.

[38] R. S. Riley, D. Rowe, and L. M. Fisher, ‘Clinical utilization of the international normalized

ratio (INR)', *J. Clin. Lab. Anal.*, vol. 14, no. 3, pp. 101–114, 2000.

[39] S. Tesema, S. Kebamo, and B. Geleta, 'Pharmacogenetics and Pharmacogenomics: Applications in Drug Development and Therapeutics', *EC Pharmacol. Toxicol.*, vol. 2, no. November, pp. 165–186, 2016.

[40] L. Yan and R. A. Beckman, 'Pharmacogenetics and pharmacogenomics in oncology therapeutic antibody development', *Biotechniques*, vol. 39, no. October, pp. 565–568, 2005.

[41] W. Sukhumsirichart, 'Polymorphisms', *Geomicrobiol. Journal16S rRNA gene Seq. Bact. Identif. diagnostic Lab.*, vol. 2007, pp. 1–8, 2007.

[42] R. Karki, D. Pandya, R. C. Elston, and C. Ferlini, 'Defining "mutation" and "polymorphism" in the era of personal genomics', *BMC Med. Genomics*, vol. 8, no. 1, pp. 1–7, 2015.

[43] D. Baralle and M. Baralle, 'Splicing in action: assessing disease causing sequence changes', pp. 737–748, 2005.

[44] E. J. Mucaki, B. C. Shirley, and P. K. Rogan, 'Expression Changes Con fi rm Genomic Variants Predicted to Result in Allele-Speci fi c , Alternative mRNA Splicing', vol. 11, no. March, pp. 1–16, 2020.

[45] D. Chu and L. Wei, 'Nonsynonymous , synonymous and nonsense mutations in human cancer-related genes undergo stronger purifying selections than expectation', no. 19, pp. 1–12, 2019.

[46] J. Valca, F. Supek, and E. Crg, 'Theory Synonymous Mutations Frequently Act as Driver Mutations in Human Cancers', 2014.

[47] T. Torgerson and H. Ochs, *Chapter 3. Genetics of Primary Immune Deficiencies*. Elsevier Inc., 2014.

[48] B. S. Shastry, 'SNP alleles in human disease and evolution', *J. Hum. Genet.*, vol. 47, no. 11, pp. 561–566, 2002.

[49] A. C. Syvanen, 'Toward genome-wide snp genotyping', *Nat. Genet.*, vol. 37, no. 6S, pp. S5–S10, 2005.

[50] L. Su *et al.*, 'Research on single nucleotide polymorphisms interaction detection from network perspective', *PLoS One*, vol. 10, no. 3, pp. 1–19, 2015.

[51] M. Gaudet, A.-G. Fara, I. Beritognolo, and M. Sabatti, 'Single nucleotide polymorphisms, methods in molecular biology', *Single Nucleotide Polymorphisms, methods Mol. Biol.*, vol. 578, no. 4, pp. 73–91, 2009.

[52] C. Zambon *et al.*, 'VKORC1, CYP2C9 and CYP4F2 genetic-based algorithm for warfarin dosing: an Italian retrospective study', pp. 15–25, 2011.

[53] M. Doo and Y. Kim, 'Review Article Obesity : Interactions of Genome and Nutrients Intake', vol. 20, no. December 2014, pp. 1–7, 2015.

[54] A-S Medication Solutions, 'WARFARIN SODIUM- warfarin tablet', 2017.

[55] K. Shaw *et al.*, 'Clinical Practice Recommendations on Genetic Testing of CYP2C9 and VKORC1 Variants in Warfarin Therapy', *Ther. Drug Monit.*, vol. 37, no. 4, pp. 428–436, 2015.

[56] M. Johnson, C. Richard, R. Bogdan, and R. Kidd, ' Warfarin Dosing in a Patient with CYP2C9*3*3 and VKORC1-1639 AA Genotypes ', *Case Rep. Genet.*, vol. 2014, pp. 1–4, 2014.

[57] J. Du, Z. Zhang, Y. Ge, J. Zhen, J. Leng, and J. Wang, 'VKORC1 and CD-14 genetic polymorphisms associate with susceptibility to cardiovascular and cerebrovascular diseases',

Int. J. Clin. Exp. Med., vol. 8, no. 11, pp. 20444–20453, 2015.

[58] B. Askari, E. Khaleqsefat, D. Khalafkhani, M. Khalaj-Kondori, K. Khademvatan, and H. Soraya, 'Study on a novel polymorphism in the VKORC1 promoter region using bioinformatic tools and warfarin dosing data', *Thromb. Res.*, vol. 158, no. May, pp. 76–78, 2017.

[59] L. Bodin, J. Perdu, M. Diry, M. H. Horellou, and M. A. Loriot, 'Multiple genetic alterations in vitamin K epoxide reductase complex subunit 1 gene (VKORC1) can explain the high dose requirement during oral anticoagulation in humans', *J. Thromb. Haemost.*, vol. 6, no. 8, pp. 1436–1439, 2008.

[60] A. Ašić *et al.*, 'Population study of thrombophilic markers and pharmacogenetic markers of warfarin prevalence in Bosnia and Herzegovina', *Croat. Med. J.*, vol. 60, no. 3, pp. 212–220, 2019.

[61] L. Yang, W. Ge, F. Yu, and H. Zhu, 'Impact of VKORC1 gene polymorphism on interindividual and interethnic warfarin dosage requirement- A systematic review and meta analysis', *Thromb. Res.*, vol. 125, no. 4, pp. e159–e166, 2010.

[62] J. Zhang, Z. Chen, and C. Chen, 'Impact of CYP2C9, VKORC1 and CYP4F2 genetic polymorphisms on maintenance warfarin dosage in Han-Chinese patients: A systematic review and meta-analysis', *Meta Gene*, vol. 9, pp. 197–209, 2016.

[63] K. Kosaki *et al.*, '1173C>T polymorphism in VKORC1 modulates the required warfarin dose', *Pediatr. Cardiol.*, vol. 27, no. 6, pp. 685–688, 2006.

[64] K. P. Balbino, H. H. M. Hermsdorff, and J. Bressan, 'Polymorphism related to cardiovascular risk in hemodialysis subjects: a systematic review', *J. Bras. Nefrol.*, vol. 40, no. 2, pp. 179–192, 2018.

[65] M. Cini *et al.*, 'A new warfarin dosing algorithm including VKORC1 3730 G > A polymorphism: Comparison with results obtained by other published algorithms', *Eur. J. Clin. Pharmacol.*, vol. 68, no. 8, pp. 1167–1174, 2012.

[66] N. A. Limdi *et al.*, 'Warfarin pharmacogenetics: A single VKORC1 polymorphism is predictive of dose across 3 racial groups', *Blood*, vol. 115, no. 18, pp. 3827–3834, 2010.

[67] N. Limdi *et al.*, 'Influence of CYP2C9 and VKORC1 on warfarin dose, anticoagulation attainment and maintenance among European American and African Americans', *Bone*, vol. 23, no. 1, pp. 1–7, 2012.

[68] T. Li *et al.*, 'Polymorphisms in the VKORC1 gene are strongly associated with warfarin dosage requirements in patients receiving anticoagulation', *J. Med. Genet.*, vol. 43, no. 9, pp. 740–744, 2006.

[69] C. Sipeky *et al.*, 'Vitamin K epoxide reductase complex 1 (VKORC1) haplotypes in healthy Hungarian and Roma population samples', *Pharmacogenomics*, vol. 10, no. 6, pp. 1026–1032, 2009.

[70] L. N. Al-Eitan, A. Y. Almasri, and R. H. Khasawneh, 'Impact of CYP2C9 and VKORC1 polymorphisms on warfarin sensitivity and responsiveness in Jordanian cardiovascular patients during the initiation therapy', *Genes (Basel).*, vol. 9, no. 12, 2018.

[71] M. H. Alrashid, A. Al-Serri, S. H. Alshemmari, P. Koshi, and S. A. Al-Bustan, 'Association of Genetic Polymorphisms in the VKORC1 and CYP2C9 Genes with Warfarin Dosage in a Group of Kuwaiti Individuals', *Mol. Diagnosis Ther.*, vol. 20, no. 2, pp. 183–190, 2016.

[72] S. E. John and S. C. John, 'Pharmacogenomics Genetic variants associated with warfarin', 2017.

[73] R. Liu, J. Cao, Q. Zhang, X. M. Shi, X. D. Pan, and R. Dong, 'Clinical and genetic factors

associated with warfarin maintenance dose in northern Chinese patients with mechanical heart valve replacement', *Med. (United States)*, vol. 96, no. 2, 2017.

[74] Z. N. Al-Mahayri *et al.*, 'Vkorc1 variants as significant predictors of Warfarin dose in Emiratis', *Pharmgenomics. Pers. Med.*, vol. 12, pp. 47–57, 2019.

[75] I. G. Asiimwe *et al.*, 'Genetic Factors Influencing Warfarin Dose in Black-African Patients: A Systematic Review and Meta-Analysis', *Clin. Pharmacol. Ther.*, vol. 107, no. 6, pp. 1420–1433, 2020.

[76] D. Van Boovena *et al.*, 'Cytochrome P450 2C9-CYP2C9', *Bone*, vol. 23, no. 1, pp. 1–7, 2010.

[77] A. Lazar, D. Tomalik-Scharte, and U. Fuhr, 'Chapter 13 Applications of genotyping and phenotyping for clinically-relevant polymorphisms of drug metabolizing enzymes and drug transporters', *Handb. Anal. Sep.*, vol. 5, pp. 321–353, 2004.

[78] J. D. Lindh, L. Holm, M. L. Andersson, and A. Rane, 'Influence of CYP2C9 genotype on warfarin dose requirements-a systematic review and meta-analysis', *Eur. J. Clin. Pharmacol.*, vol. 65, no. 4, pp. 365–375, 2009.

[79] P. Weeke and D. M. Roden, 'Pharmacogenomics and cardiovascular disease', *Curr. Cardiol. Rep.*, vol. 15, no. 7, pp. 1–16, 2013.

[80] S. A. Scott, R. Khasawneh, I. Peter, R. Kornreich, and R. J. Desnick, 'Combined CYP2C9, VKORC1 and CYP4F2 frequencies among racial and ethnic groups', *Pharmacogenomics*, vol. 11, no. 6, pp. 781–791, 2010.

[81] O. Singh, E. Sandanaraj, K. Subramanian, L. H. Lee, and B. Chowbay, 'Influence of CYP4F2 rs2108622 (V433M) on warfarin dose requirement in asian patients', *Drug Metab. Pharmacokinet.*, vol. 26, no. 2, pp. 130–136, 2011.

[82] T. Zhang, K. Yu, and X. Li, 'Cytochrome P450 family 4 subfamily F member 2 (CYP4F2) rs1558139, rs2108622 polymorphisms and susceptibility to several cardiovascular and cerebrovascular diseases', *BMC Cardiovasc. Disord.*, vol. 18, no. 1, pp. 1–10, 2018.

[83] V. Tatarunas, N. Kupstyte, R. Zaliunas, A. Giedraitiene, and V. Lesauskaite, 'The impact of clinical and genetic factors on ticαelor and clopidogrel antiplatelet therapy', *Pharmacogenomics*, vol. 18, no. 10, pp. 969–979, 2017.

[84] H. Geng, B. Li, Y. Wang, and L. Wang, 'Association between the CYP4F2 Gene rs1558139 and rs2108622 Polymorphisms and Hypertension: A Meta-Analysis', *Genet. Test. Mol. Biomarkers*, vol. 23, no. 5, pp. 342–347, 2019.

[85] W. Y. Kim *et al.*, 'Identification of novel CYP4F2 genetic variants exhibiting decreased catalytic activity in the conversion of arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE)', *Prostaglandins Leukot. Essent. Fat. Acids*, vol. 131, pp. 6–13, 2018.

[86] S. Sirotina *et al.*, 'A Novel Polymorphism in the Promoter of the CYP4A11 Gene Is Associated with Susceptibility to Coronary Artery Disease', *Dis. Markers*, vol. 2018, 2018.

[87] Peter Jordan, 'Großgliederung Europas nach kulturräumlichen Kriterien', *Eur. Reg.*, vol. 13, no. 4, pp. 162–173, 2005.

[88] K. Mirzaev *et al.*, 'Interethnic differences in the prevalence of main cardiovascular pharmacogenetic biomarkers', *Pharmacogenomics*, vol. 21, no. 10, pp. 677–694, 2020.

[89] M. Andreas *et al.*, 'The VKORC1 polymorphism rs9923231 is associated with aneurysms of the ascending aorta in an Austrian population', *Thromb. Res.*, vol. 152, pp. 41–43, 2017.

[90] M. Weger *et al.*, 'Role of the vitamin K epoxide reductase complex subunit 1 (VKORC1) - 1639G>A gene polymorphism in patients with retinal vein occlusion', *Curr. Eye Res.*, vol. 38,

no. 12, pp. 1278–1282, 2013.

[91] D. Mandić, S. Mandić, V. Horvat, M. Samardžija, and M. Samardžija, 'Vitamin K epoxide reductase complex 1 (VKORC1) gene polymorphisms in population of eastern Croatia', *Coll. Antropol.*, vol. 37, no. 4, pp. 1321–1326, 2013.

[92] W. Matthias *et al.*, 'Functional promoter polymorphism in the VKORC1 gene is no major genetic determinant for coronary heart disease in Northern Germans', *Thromb. Haemost.*, vol. 98, no. 2, pp. 406–412, 2007.

[93] C. Geisen *et al.*, 'VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation', *Thromb. Haemost.*, vol. 94, no. 4, pp. 773–779, 2005.

[94] M. Watzka, P. Westhofen, M. Hass, M. Marinova, B. Pötzsch, and J. Oldenburg, 'Polymorphisms in VKORC1 and GGCX are not major genetic determinants of vitamin K-dependent coagulation factor activity in Western Germans', *Thromb. Haemost.*, vol. 102, no. 2, pp. 418–420, 2009.

[95] G. Spohn, C. Geisen, B. Luxembourg, K. Sittinger, E. Seifried, and H. Bönig, 'Erratum: Validation of a rapid and inexpensive allele-specific amplification (ASA)-PCR genotyping assay for vitamin K antagonist pharmacogenomics (Molecular Diagnosis and Therapy (2011) 15:1 (513-519))', *Mol. Diagnosis Ther.*, vol. 15, no. 3, pp. 142–144, 2011.

[96] D. Werner *et al.*, 'Pharmacogenetic characteristics of patients with complicated phenprocoumon dosing', *Eur. J. Clin. Pharmacol.*, vol. 65, no. 8, pp. 783–788, 2009.

[97] J. Wolkanin-Bartnik, H. Pogorzelska, M. Szperl, A. Bartnik, J. Koziarek, and Z. T. Bilinska, 'Impact of genetic and clinical factors on dose requirements and quality of anticoagulation therapy in Polish patients receiving acenocoumarol: Dosing calculation algorithm', *Pharmacogenet. Genomics*, vol. 23, no. 11, pp. 611–618, 2013.

[98] Ľ. Krajčíová, R. Petrovič, Ľ. Déžiová, J. Chandoga, and P. Turčáni, 'Frequency of selected single nucleotide polymorphisms influencing the warfarin pharmacogenetics in Slovak population', *Eur. J. Haematol.*, vol. 93, no. 4, pp. 320–328, 2014.

[99] G. Ragia, S. Marousi, J. Ellul, V. G. Manolopoulos, and A. Tavridou, 'Association of functional VKORC1 promoter polymorphism with occurrence and clinical aspects of ischemic stroke in a Greek population', *Dis. Markers*, vol. 35, no. 6, pp. 641–646, 2013.

[100] A. Christodoulou, E. Bagli, M. Gazouli, M. M. Moschos, and G. Kitsos, 'Genetic polymorphisms associated with the prevalence of retinal vein occlusion in a Greek population', *Int. Ophthalmol.*, vol. 39, no. 11, pp. 2637–2648, 2019.

[101] A. Tavridou *et al.*, 'Association of VKORC1 -1639 G>A polymorphism with carotid intima-media thickness in type 2 diabetes mellitus', *Diabetes Res. Clin. Pract.*, vol. 94, no. 2, pp. 236–241, 2011.

[102] K. A. Nestorovska *et al.*, 'Distribution of the most common genetic variants associated with a variable drug response in the population of the Republic of Macedonia', *Balk. J. Med. Genet.*, vol. 17, no. 2, pp. 5–14, 2014.

[103] I. Groza *et al.*, 'VKORC1-1639 G>A polymorphism and the risk of non-variceal upper gastrointestinal bleeding', *J. Gastrointest. Liver Dis.*, vol. 26, no. 1, pp. 13–18, 2017.

[104] A. D. Buzoianu, A. P. Trifa, D. F. Mureşanu, and S. Crişan, 'Analysis of CYP2C9*2, CYP2C9*3 and VKORC1 -1639 G>A polymorphisms in a population from South-Eastern Europe', *J. Cell. Mol. Med.*, vol. 16, no. 12, pp. 2919–2924, 2012.

[105] Ş. C. Vesa, A. P. Trifa, S. Crişan, and A. D. Buzoianu, 'VKORC1 -1639 G>A Polymorphism

in Romanian Patients with Deep Vein Thrombosis', *Clin. Appl. Thromb.*, vol. 22, no. 8, pp. 760–764, 2016.

[106] B. D. Taşkın *et al.*, *The effect of CYP2C9 and VKORC1 genetic polymorphisms on warfarin dose requirements in a pediatric population*, vol. 16, no. 10. 2016.

[107] F. Kutluturk *et al.*, 'Association between Vitamin K Epoxide Reductase (VKORC1) -1639G>A Polymorphism and Osteoporosis in Postmenopausal Women', *Endocrine, Metab. Immune Disord. - Drug Targets*, 2018.

[108] H. D. Demir, H. Ortak, Ş. Şahin, Ö. Ateş, I. Benli, and A. Inanir, 'VKORC1 C1173T and VKORC1 G-1639A gene polymorphisms in turkish Behçet's patients with ocular and non-ocular involvement', *Ophthalmic Genet.*, vol. 35, no. 1, pp. 7–11, 2014.

[109] H. Ortak, E. Söğüt, H. Demir, A. Ardagil, I. Benli, and S. Sahin, 'Predictive value of the vitamin K epoxide reductase complex subunit 1 G-1639A and C1173T single nucleotide polymorphisms in retinal vein occlusion', *Clin. Exp. Ophthalmol.*, vol. 40, no. 7, pp. 743–748, 2012.

[110] U. Berber *et al.*, 'Genetic Polymorphism of VKORC1-1639 in Children With Intracranial Hemorrhage Due to Vitamin K Deficiency', *Clin. Appl. Thromb.*, vol. 24, no. 9_suppl, pp. 89S-93S, 2018.

[111] C. Ozbayer *et al.*, 'Lack of association between type 2 diabetes and the 3673G/A and 9041G/a gene variants of vitamin K epoxide reductase complex subunit 1 (VKORC1)', *Int. J. Vitam. Nutr. Res.*, vol. 86, no. 3–4, pp. 133–139, 2016.

[112] D. T. Cosan *et al.*, 'Susceptiveness of Vitamin K epOxide Reductase Complex Subunit 1 Gene Polymorphism in Essential Hypertension', *Genet. Test. Mol. Biomarkers*, vol. 21, no. 5, pp. 292–297, 2017.

[113] L. Bodin *et al.*, 'Genotypes As Determinants of Acenocoumarol Sensitivity', *Blood*, vol. 9, no. 1, pp. 135–140, 2012.

[114] D. Hadjipanagi, S. Chrysanthou, K. Voskarides, and C. Deltas, 'Genetic polymorphisms in warfarin and tacrolimus-related genes VKORC1, CYP2C9 and CYP3A5 in the Greek-Cypriot population', *BMC Res. Notes*, vol. 7, no. 1, pp. 3–7, 2014.

[115] S. Misasi *et al.*, 'VKORC1 and CYP2C9 polymorphisms related to adverse events in case-control cohort of anticoagulated patients', *Med. (United States)*, vol. 95, no. 52, pp. 1–7, 2016.

[116] G. Suarez-Kurtz *et al.*, 'VKORC1 polymorphisms in Brazilians: Comparison with the Portuguese and Portuguese-speaking Africans and pharmacogenetic implications', *Pharmacogenomics*, vol. 11, no. 9, pp. 1257–1267, 2010.

[117] C. C. Branco, T. Pereirinha, R. Cabral, P. R. Pacheco, and L. Mota-Vieira, 'Thrombotic genetic risk factors and warfarin pharmacogenetic variants in São Miguel's healthy population (Azores)', *Thromb. J.*, vol. 7, pp. 1–9, 2009.

[118] A. Augustyn *et al.*, 'Turkic peoples', *Encyclopædia Britannica*. Encyclopædia Britannica, inc., 2020.

[119] A. D. Ciubean *et al.*, 'Polymorphisms of FDPS, LRP5, SOST and VKORC1 genes and their relation with osteoporosis in postmenopausal Romanian women', *PLoS One*, vol. 14, no. 11, pp. 1–11, 2019.

[120] C. Verstuyft *et al.*, 'A Pharmacokinetic-pharmacodynamic model for predicting the impact of CYP2C9 and VKORC1 polymorphisms on fluindione and acenocoumarol during induction therapy', *Clin. Pharmacokinet.*, vol. 51, no. 1, pp. 41–53, 2012.

[121] K. Lacut *et al.*, 'Vitamin K epoxide reductase genetic polymorphism is associated with venous

thromboembolism: Results from the EDITH Study', *J. Thromb. Haemost.*, vol. 5, no. 10, pp. 2020–2024, 2007.

[122] M. Teichert *et al.*, 'Vitamin K epoxide reductase complex subunit 1 (VKORC1) polymorphism and aortic calcification: The Rotterdam study', *Arterioscler. Thromb. Vasc. Biol.*, vol. 28, no. 4, pp. 771–776, 2008.

[123] G. D. Andrea *et al.*, 'A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin', *Blood*, vol. 105, no. 2, pp. 645–649, 2005.

[124] T. Dujic, K. Zhou, L. A. Donnelly, G. Leese, C. N. A. Palmer, and E. R. Pearson, 'Interaction between variants in the CYP2C9 and POR genes and the risk of sulfonylurea-induced hypoglycaemia: A GoDARTS Study', 2017.

[125] N. Božina, P. Granić, Z. Lalić, I. Tramišak, M. Lovrić, and A. Stavljenić-Rukavina, 'Genetic polymorphisms of cytochromes P450: CYP2C9, CYP2C19, and CYP2D6 in Croatian population', *Croat. Med. J.*, vol. 44, no. 4, pp. 425–428, 2003.

[126] A. C. Allabi, J. L. Gala, J. P. Desager, M. Heusterspreute, and Y. Horsmans, 'Genetic polymorphisms of CYP2C9 and CYP2C19 in the Beninese and Belgian populations', *Br. J. Clin. Pharmacol.*, vol. 56, no. 6, pp. 653–657, 2003.

[127] S. P. Abdullaev *et al.*, 'Clinically relevant pharmacogenetic markers in Tatars and Balkars', *Mol. Biol. Rep.*, vol. 47, no. 5, pp. 3377–3387, 2020.

[128] G. Korytina, O. Kochetova, L. Akhmadishina, E. Viktorova, and T. Victorova, 'Polymorphisms of cytochrome P450 genes in three ethnic groups from Russia', *Balkan Med. J.*, vol. 29, no. 3, pp. 252–260, 2012.

[129] K. Mirzaev *et al.*, 'Genetic Polymorphisms of Cytochrome P450 Enzymes and Transport Proteins in a Russian Population and Three Ethnic Groups of Dagestan', *Genet. Test. Mol. Biomarkers*, vol. 21, no. 12, pp. 747–753, 2017.

[130] O. Gra *et al.*, 'Microarray-Based Detection of CYP1A1, CYP2C9, CYP2C19, CYP2D6, GSTT1, GSTM1, MTHFR, MTRR, NQO1, NAT2, HLA-DQA1, and AB0 Allele Frequencies in Native Russians', *J. Chem. Inf. Model.*, vol. 53, no. 9, pp. 1689–1699, 2010.

[131] O. Gra *et al.*, 'Polymorphisms in xenobiotic-metabolizing genes and the risk of chronic lymphocytic leukemia and non-Hodgkin's lymphoma in adult Russian patients', *Am. J. Hematol.*, vol. 83, no. 4, pp. 279–287, 2008.

[132] E. A. Gaikovitch *et al.*, 'Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population', *Eur. J. Clin. Pharmacol.*, vol. 59, no. 4, pp. 303–312, 2003.

[133] V. N. Pimenoff *et al.*, 'Similarity in recombination rate and linkage disequilibrium at CYP2C and CYP2D cytochrome P450 gene regions among Europeans indicates signs of selection and no advantage of using tagSNPs in population isolates', *Pharmacogenet. Genomics*, vol. 22, no. 12, pp. 846–857, 2012.

[134] R. S. Pedersen *et al.*, 'Linkage disequilibrium between the CYP2C19*17 allele and wildtype CYP2C8 and CYP2C9 alleles: Identification of CYP2C haplotypes in healthy Nordic populations', *Eur. J. Clin. Pharmacol.*, vol. 66, no. 12, pp. 1199–1205, 2010.

[135] D. Kaur-Knudsen, B. G. Nordestgaard, and S. E. Bojesen, 'CYP2C9 genotype does not affect risk of tobacco-related cancer in the general population', *Cancer Epidemiol.*, vol. 34, no. 2, pp. 178–183, 2010.

[136] R. S. Pedersen, C. Verstuyft, L. Becquemont, P. Jaillon, and K. Brøsen, 'Cytochrome P4502C9

(CYP2C9) Genotypes in a Nordic Population in Denmark', *Basic Clin. Pharmacol. Toxicol.*, vol. 94, no. 3, pp. 151–152, 2004.

[137] C. Kragelund *et al.*, 'Polymorphic drug metabolizing CYP-enzymes - A pathogenic factor in oral lichen planus?', *J. Oral Pathol. Med.*, vol. 38, no. 1, pp. 63–71, 2009.

[138] M. Niemi, I. Cascorbi, R. Timm, H. K. Kroemer, P. J. Neuvonen, and K. T. Kivistö, 'Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes', *Clin. Pharmacol. Ther.*, vol. 72, no. 3, pp. 326–332, 2002.

[139] T. Karonen, J. Laitila, M. Niemi, P. J. Neuvonen, and J. T. Backman, 'Fluconazole but not the CYP3A4 inhibitor, itraconazole, increases zafirlukast plasma concentrations', *Eur. J. Clin. Pharmacol.*, vol. 68, no. 5, pp. 681–688, 2012.

[140] J. Hilli, A. Rane, S. Lundgren, L. Bertilsson, and K. Laine, 'Genetic polymorphism of cytochrome P450s and P-glycoprotein in the Finnish population', *Fundam. Clin. Pharmacol.*, vol. 21, no. 4, pp. 379–386, 2007.

[141] Ü. Yasar *et al.*, 'Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP2C9 genotype', *Clin. Pharmacol. Ther.*, vol. 71, no. 1, pp. 89–98, 2002.

[142] Ü. Yasar, E. Eliasson, M. L. Dahl, I. Johansson, M. Ingelman-Sundberg, and F. Sjöqvist, 'Validation of methods for CYP2C9 genotyping: Frequencies of mutant alleles in a Swedish population', *Biochem. Biophys. Res. Commun.*, vol. 254, no. 3, pp. 628–631, 1999.

[143] A. M. Bennet, E. Eliasson, S. Lundgren, and C. P. Cyp, 'Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction', *Pharmacogenetics*, pp. 715–720, 2003.

[144] M. Funk *et al.*, 'Influence of Cytochrome P450 2C9*2 and 2C9*3 Variants on the Risk of Ischemic Stroke: A Cross-sectional Case–Control Study.', *Clin. Chem.*, vol. 51, no. 9, pp. 1713–1716, 2005.

[145] E. Topić, M. Štefanović, and M. Samardžija, 'Association between the CYP2C9 polymorphism and the drug metabolism phenotype', *Clin. Chem. Lab. Med.*, vol. 42, no. 1, pp. 72–78, 2004.

[146] L. Ganoci, T. Božina, N. Mirošević Skvrce, M. Lovrić, P. Mas, and N. Božina, 'Genetic polymorphisms of cytochrome P450 enzymes: CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 in the Croatian population', *Drug Metab. Pers. Ther.*, vol. 32, no. 1, pp. 11–21, 2017.

[147] N. Miroševic Skvrce, N. Božina, L. Zibar, I. Barišic, L. Pejnovic, and V. Macolic Šarinic, 'CYP2C9 and ABCG2 polymorphisms as risk factors for developing adverse drug reactions in renal transplant patients taking fluvastatin: A case-control study', *Pharmacogenomics*, vol. 14, no. 12, pp. 1419–1431, 2013.

[148] H. Buzková *et al.*, 'Lipid-lowering effect of fluvastatin in relation to cytochrome P450 2C9 variant alleles frequently distributed in the Czech population', *Med. Sci. Monit.*, vol. 18, no. 8, pp. 512–517, 2012.

[149] A. Rudolph *et al.*, 'Modification of menopausal hormone therapy-associated colorectal cancer risk by polymorphisms in sex steroid signaling, metabolism and transport related genes', *Endocr. Relat. Cancer*, vol. 18, no. 3, pp. 371–384, 2011.

[150] J. Bolbrinker *et al.*, 'Role of CYP2C9 genetic variants for salt sensitivity and the regulation of the renin-angiotensin-aldosterone system in normotensive men', *J. Hypertens.*, vol. 29, no. 1, pp. 56–61, 2011.

[151] A. Gasse, M. Vennemann, H. Köhler, and J. Schürenkamp, 'Toxicogenetic analysis of Δ9-THC-metabolizing enzymes', *Int. J. Legal Med.*, 2020.

[152] S. Vogl, R. W. Lutz, G. Schönfelder, and W. K. Lutz, 'CYP2C9 genotype vs. metabolic phenotype for individual drug dosing-A correlation analysis using flurbiprofen as probe drug',

PLoS One, vol. 10, no. 3, 2015.

[153] R. Varnai, C. Sipeky, L. Nagy, S. Balogh, and B. Melegh, 'CYP2C9 and VKORC1 in therapeutic dosing and safety of acenocoumarol treatment: implication for clinical practice in Hungary', *Environ. Toxicol. Pharmacol.*, vol. 56, pp. 282–289, 2017.

[154] M. Makowska, B. Smolarz, M. Bryś, E. Forma, and H. Romanowicz, 'An association between the rs1799853 and rs1057910 polymorphisms of CYP2C9, the rs4244285 polymorphism of CYP2C19 and the prevalence rates of drug-resistant epilepsy in children', *Int. J. Neurosci.*, vol. 7454, 2020.

[155] M. Karaniewicz-Ada, M. Uczak, and F. Główka, 'Pharmacokinetic studies of enantiomers of ibuprofen and its chiral metabolites in humans with different variants of genes coding CYP2C8 and CYP2C9 isoenzymes', *Xenobiotica*, vol. 39, no. 6, pp. 476–485, 2009.

[156] K. L, D. L, P. R, L. J, T. P, and C. J, 'Frequencies of polymorphisms in CYP2C9 and VKORC1 genes infl uencing warfarin metabolism in Slovak population: implication for clinical practice', *Bratisl. lek??rske List.*, vol. 116, no. 5, pp. 296–301, 2014.

[157] W. R. Wolowich, R. Greif, M. Kleine-Brueggeney, W. Bernhard, and L. Theiler, 'Minimal Physiologically Based Pharmacokinetic Model of Intravenously and Orally Administered Delta-9-Tetrahydrocannabinol in Healthy Volunteers', *Eur. J. Drug Metab. Pharmacokinet.*, vol. 44, no. 5, pp. 691–711, 2019.

[158] S. Semiz *et al.*, 'Analysis of CYP2C9*2, CYP2C19*2, and CYP2D6*4 polymorphisms in patients with type 2 diabetes mellitus', *Bosn. J. Basic Med. Sci.*, p. 960, 2010.

[159] K. Arvanitidis *et al.*, 'Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population', *Fundam. Clin. Pharmacol.*, vol. 21, no. 4, pp. 419–426, 2007.

[160] V. Krasniqi, A. Dimovski, H. Q. Bytyqi, A. Eftimov, L. Šimičevic, and N. Božina, 'Genetic polymorphisms of CYP2C9, CYP2C19, and CYP3A5 in Kosovar population', *Arh. Hig. Rada Toksikol.*, vol. 68, no. 3, pp. 180–184, 2017.

[161] K. Jakovski, A. K. Nestorovska, N. Labacevski, and A. J. Dimovski, 'Characterization of the most common CYP2C9 and CYP2C19 allelic variants in the population from the Republic of Macedonia', *Pharmazie*, vol. 68, no. 11, pp. 893–898, 2013.

[162] A. Kapedanovska Nestorovska *et al.*, 'AKR1D1∗36 C>T (rs1872930) allelic variant is associated with variability of the CYP2C9 genotype predicted pharmacokinetics of ibuprofen enantiomers - A pilot study in healthy volunteers', *Acta Pharm.*, vol. 69, no. 3, pp. 399–412, 2019.

[163] I. Skadrić and O. Stojković, 'Defining screening panel of functional variants of CYP1A1, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 genes in Serbian population', *Int. J. Legal Med.*, 2019.

[164] A. Sükrü Aynacioglu *et al.*, 'Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin', *Br. J. Clin. Pharmacol.*, vol. 48, no. 3, pp. 409–415, 1999.

[165] M. T. Goktas *et al.*, 'Decreased Activity and Genetic Polymorphisms of CYP2C19 in Behçet's Disease', *Basic Clin. Pharmacol. Toxicol.*, vol. 121, no. 4, pp. 266–271, 2017.

[166] M. O. Babaoglu *et al.*, 'CYP2C9 genetic variants and losartan oxidation in a Turkish population', *Eur. J. Clin. Pharmacol.*, vol. 60, no. 5, pp. 337–342, 2004.

[167] M. T. Goktaş *et al.*, 'Lower CYP2C9 activity in Turkish patients with Behçet's disease compared to healthy subjects: A down-regulation due to inflammation?', *Eur. J. Clin.*

Pharmacol., vol. 71, no. 10, pp. 1223–1228, 2015.

[168] M. Arici and G. Özhan, 'CYP2C9, CYPC19 and CYP2D6 gene profiles and gene susceptibility to drug response and toxicity in Turkish population', *Saudi Pharm. J.*, vol. 25, no. 3, pp. 376–380, 2017.

[169] M. Seven, B. Batar, S. Unal, G. Yesil, A. Yuksel, and M. Guven, 'The effect of genetic polymorphisms of cytochrome P450 CYP2C9, CYP2C19, and CYP2D6 on drug-resistant epilepsy in Turkish children', *Mol. Diagnosis Ther.*, vol. 18, no. 2, pp. 229–236, 2014.

[170] I. Ö. Barlas *et al.*, 'Harnessing knowledge on very important pharmacogenes CYP2C9 and CYP2C19 variation for precision medicine in resource-limited global conflict zones', *Omi. A J. Integr. Biol.*, vol. 20, no. 10, pp. 604–609, 2016.

[171] Ö. Düzovali *et al.*, 'Glutathione S-Transferases CYP2C9 and CYP2C19 Polymorphisms in Turkish Children with Cancer', no. January, pp. 23–26, 2008.

[172] B. Ercan, L. Ayaz, D. Çiçek, and L. Tamer, 'Role of CYP2C9 and CYP2C19 polymorphisms in patients with atherosclerosis', *Cell Biochem. Funct.*, vol. 26, pp. 320–328, 2008.

[173] G. P. Aithal, C. P. Day, P. J. L. Kesteven, and A. K. Daly, 'Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications', *Lancet*, vol. 353, no. 9154, pp. 717–719, 1999.

[174] R. Howard *et al.*, 'Genotyping for CYP2C9 and VKORC1 alleles by a novel point of care assay with HyBeacon® probes', *Clin. Chim. Acta*, vol. 412, no. 23–24, pp. 2063–2069, 2011.

[175] C. O. Musumba *et al.*, 'CYP2C19*17 gain-of-function polymorphism is associated with peptic ulcer disease', *Clin. Pharmacol. Ther.*, vol. 93, no. 2, pp. 195–203, 2013.

[176] J. Q. Yang *et al.*, 'Frequency of cytochrome P450 2C9 allelic variants in the Chinese and French populations', *Fundam. Clin. Pharmacol.*, vol. 17, no. 3, pp. 373–376, 2003.

[177] S. Chhun *et al.*, 'The Cytochrome P-450 2C9/2C19 but Not the ABCB1 Genetic Polymorphism May Be Associated With the Liver Cytochrome 3A4 Induction by Phenytoin', vol. 32, no. 3, pp. 429–431, 2012.

[178] P. Shahabi *et al.*, 'Influence of genetic variations on levels of inflammatory markers of healthy subjects at baseline and one week after clopidogrel therapy; results of a preliminary study.', *Int. J. Mol. Sci.*, vol. 14, no. 8, pp. 16402–16413, 2013.

[179] T. M. Bosch, V. D. Doodeman, P. H. M. Smits, I. Meijerman, J. H. M. Schellens, and J. H. Beijnen, 'Pharmacogenetic screening for polymorphisms in drug-metabolizing enzymes and drug transporters in a Dutch population', *Mol. Diagnosis Ther.*, vol. 10, no. 3, pp. 175–185, 2006.

[180] F. Carano *et al.*, 'Genetic variability of CYP2D6, CYP2B6, CYP2C9 and CYP2C19 genes across the Italian Peninsula', *Ann. Hum. Biol.*, vol. 45, no. 1, pp. 66–71, 2018.

[181] D. Caccamo *et al.*, 'Xenobiotic sensor- and metabolism-related gene variants in environmental sensitivity-related illnesses: A survey on the Italian population', *Oxid. Med. Cell. Longev.*, vol. 2013, 2013.

[182] M. G. Scordo, A. P. Caputi, C. D'Arrigo, G. Fava, and E. Spina, 'Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population', *Pharmacol. Res.*, vol. 50, no. 2, pp. 195–200, 2004.

[183] A. Figueiras *et al.*, 'CYP2C9 variants as a risk modifier of NSAID-related gastrointestinal bleeding: A case-control study', *Pharmacogenet. Genomics*, vol. 26, no. 2, pp. 66–73, 2016.

[184] M. Falzoi, A. Mossa, E. Congeddu, L. Saba, and L. Pani, 'Multiplex genotyping of CYP3A4,

CYP3A5, CYP2C9 and CYP2C19 SNPs using MALDI-TOF mass spectrometry', pp. 1–13, 2010.

[185] C. De Luca *et al.*, 'Biological definition of multiple chemical sensitivity from redox state and cytokine profiling and not from polymorphisms of xenobiotic-metabolizing enzymes', *Toxicol. Appl. Pharmacol.*, vol. 248, no. 3, pp. 285–292, 2010.

[186] E. García-Martín, C. Martínez, J. M. Ladero, F. J. Gamito, A. Rodriguez-Lescure, and J. A. G. Agúndez, 'Influence of cytochrome P450 CYP2C9 genotypes in lung cancer risk', *Cancer Lett.*, vol. 180, no. 1, pp. 41–46, 2002.

[187] P. Zubiaur *et al.*, 'Effect of Polymorphisms in CYP2C9 and CYP2C19 on the Disposition, Safety and Metabolism of Progesterone Administrated Orally or Vaginally', *Adv. Ther.*, vol. 36, no. 10, pp. 2744–2755, 2019.

[188] T. Cabaleiro *et al.*, 'Evaluation of the Relationship between Sex, Polymorphisms in CYP2C8 and CYP2C9, and Pharmacokinetics of Angiotensin Receptor Blockers', *J. Clin. Pharmacol.*, vol. 53, no. 12, pp. 1261–1267, 2012.

[189] T. Cabaleiro *et al.*, 'Pharmacogenetics of quetiapine in healthy volunteers: Association with pharmacokinetics, pharmacodynamics, and adverse effects', *Int. Clin. Psychopharmacol.*, vol. 30, no. 2, pp. 82–88, 2015.

[190] A. M. Borobia *et al.*, 'CYP2C9 polymorphism in five autochthonous population of the same geographic area (Spanish Pyrenees)', *Pharmacol. Res.*, vol. 59, no. 2, pp. 107–111, 2009.

[191] E. García-Martín, C. Martínez, J. M. Ladero, F. J. G. Gamito, and J. A. G. Agúndez, 'High frequency of mutations related to impaired CYP2C9 metabolism in a Caucasian population', *Eur. J. Clin. Pharmacol.*, vol. 57, no. 1, pp. 47–49, 2001.

[192] P. Dorado, R. Berecz, M. J. Norberto, Ü. Yasar, M. L. Dahl, and A. LLerena, 'CYP2C9 genotypes and diclofenac metabolism in Spanish healthy volunteers', *Eur. J. Clin. Pharmacol.*, vol. 59, no. 3, pp. 221–225, 2003.

[193] P. Dorado *et al.*, 'CYP2C9 allele frequency differences between populations of Mexican-Mestizo, Mexican-Tepehuano, and Spaniards', *Pharmacogenomics J.*, vol. 11, no. 2, pp. 108–112, 2011.

[194] C. Martínez *et al.*, 'Gender and functional CYP2C and NAT2 polymorphisms determine the metabolic profile of metamizole', *Biochem. Pharmacol.*, vol. 92, no. 3, pp. 457–466, 2014.

[195] C. Martínez, E. García-Martín, G. Blanco, F. J. G. Gamito, J. M. Ladero, and J. A. G. Agúndez, 'The effect of the cytochrome P450 CYP2C8 polymorphism on the disposition of (R)-ibuprofen enantiomer in healthy subjects', *Br. J. Clin. Pharmacol.*, vol. 59, no. 1, pp. 62–68, 2005.

[196] V. Bosó *et al.*, 'Genotype and allele frequencies of drug-metabolizing enzymes and drug transporter genes affecting immunosuppressants in the Spanish white population', *Ther. Drug Monit.*, vol. 36, no. 2, pp. 159–168, 2014.

[197] M. Á. Pena *et al.*, 'Effect of Cytochrome P450 and ABCB1 Polymorphisms on Imatinib Pharmacokinetics After Single-Dose Administration to Healthy Subjects', *Clin. Drug Investig.*, vol. 40, no. 7, pp. 617–628, 2020.

[198] J. Vicente, F. González-Andrade, A. Soriano, A. Fanlo, B. Martínez-Jarreta, and B. Sinués, 'Genetic polymorphisms of CYP2C8, CYP2C9 and CYP2C19 in Ecuadorian Mestizo and Spaniard populations: A comparative study', *Mol. Biol. Rep.*, vol. 41, no. 3, pp. 1267–1272, 2014.

[199] I. Dapía *et al.*, 'Prediction models for voriconazole pharmacokinetics based on

pharmacogenetics: AN exploratory study in a Spanish population', *Int. J. Antimicrob. Agents*, vol. 54, no. 4, pp. 463–470, 2019.

[200] C. W. K. Chiang *et al.*, 'Genomic history of the Sardinian population', *Nat. Genet.*, vol. 50, no. 10, pp. 1426–1434, 2018.

[201] C. Sipeky *et al.*, 'Interethnic variability of CYP4F2 (V433M) in admixed population of Roma and Hungarians', *Environ. Toxicol. Pharmacol.*, vol. 40, no. 1, pp. 280–283, 2015.

[202] R. Sakiene *et al.*, 'CYP4F2 (rs2108622) Gene Polymorphism Association with Age-Related Macular Degeneration', *Adv. Med.*, vol. 2016, pp. 1–9, 2016.

[203] G. Gervasini *et al.*, 'Polymorphisms in CYP-mediated arachidonic acid routes affect the outcome of renal transplantation', *Eur. J. Clin. Invest.*, vol. 45, no. 10, pp. 1060–1068, 2015.