KULSOOM ZEHRA

DETERMINING THE ALLELE FREQUENCY AND GENOTYPE DISTRIBUTION OF *CYP2C9* AND *VKORC* 1 GENE POLYMORPHISM IN TURKISH CYPRIOT POPULATION

MSc. THESIS

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DEPARTMENT OF MEDICAL GENETICS

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DECLARATION

I hereby declare that all of the information in this document was gathered and presented in a manner that is compliant with academic regulations and ethical standards of behavior. I further declare that, in accordance with these rules and standards of behavior, I have properly attributed and referenced all information and outcomes that are not my own and are not unique to this work.

Kulsoom Zehra

Date:

DEDICATION

This thesis is dedicated to my dearest mother, as a token of my love, admiration, and appreciation. Without your unwavering love and support, this accomplishment would not have been possible. Your presence in my life has been an immeasurable blessing, and I am forever grateful for the incredible mother you are. My supervisor, Prof. MAHMUT ÇERKEZ ERGÖREN who has been both a mentor and a model for me to follow. To all of my family and friends that supported me and remained by my side during the challenging times.

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ABSTRACT

"DETERMINING THE ALLELE FREQUENCY AND GENOTYPE DISTRIBUTION OF *CYP2C9* AND *VKORC1* GENE POLYMORPHISM IN TURKISH CYPRIOT POPULATION"

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AIM: In this study, the genotype distribution and allele frequencies of the *CYP2C9* and *VKORC1* gene polymorphisms in the Turkish Cypriot community is examine. Turkish Cypriots, a distinct ethnic subgroup living in Cyprus, have distinctive genetic traits that may explain why their medication responses differ from those of other groups such as Africans, Americans, Greek-Cypriot, Europeans and Asians (including Chinese, Turkish, Egyptian and Japanese).

BACKGROUND: Pharmacogenetic study looks on how genetic differences affect individual drug responses. The *CYP2C9* and *VKORC1* genes are important in medication metabolism, especially for the anticoagulant warfarin. Warfarin metabolism can be affected by *CYP2C9* polymorphisms, resulting in greater drug concentrations and an increased risk of bleeding. *VKORC1* is required for vitamin K activation, which is required for the generation of clotting factors. Understanding how these genes affect medication metabolism improves personalized medicine techniques and aids in the prevention of harmful drug responses.

METHOD: Deoxyribonucleic acid (DNA) is recovered from blood samples taken from 50 Turkish Cypriots using a DNA isolation kit. A spectrophotometer is use to determine the concentration of deoxyribonucleic acid (DNA). The *CYP2C9*2 (1639G>A)* and *VKORC1 (C4302T)* mutations are genotyped with a commercial Warfarin Real-Time PCR Kit. The fluorescence signals are used to determine the genotypes after real-time PCR amplification and genotyping. To assess the Hardy-Weinberg equilibrium and compare the results to other populations, statistical analysis is performed.

RESULTS: Males in a study with 50 participants (equal gender distribution) have an average age of 45.8 years, females have an average age of 36.3 years, and the overall average age is 40.1 years. The age difference between genders is statistically significant (p=0.039). In *CYP2C9 G1639A*, the allele frequencies are 0.580 for G and 0.420 for A, and 0.860 for C and 0.140 for T in *VKORC1 C430T*. The genotype distribution and allele frequencies in the Turkish Cypriot population closely align with the anticipated genetic distribution, indicating a substantial genetic congruence within this specific demographic group. These findings shed light on the genetic make-up of Turkish Cypriots, notably in terms of gene polymorphisms, and have implications for tailored medication and understanding pharmacokinetic profiles in this community.

DISCUSSION: The distributions of genotypes and allele frequencies are examined and compared to the expected distribution. There is no significant difference between the observed and anticipated distributions. The G allele in *CYP2C9 G1639A* is more prevalent, as the C allele in *VKORC1 C430T*. Assuming that in Turkish Cypriot individuals *CYP2C9* and *VKORC1* enzymes activity is normal or near to normal so the average warfarin dosage requirement may be slightly high.

KEYWORDS: Allele frequency, Genotype distribution, Hardy-Weinberg equilibrium, *CYP2C9* gene polymorphism, *VKORC1* gene polymorphism

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LIST OF ABBREVIATION

DNA: Deoxyribonucleic acid

PCR: Polymerase chain reaction

VKAs: Vitamin K antagonists

DVT: Deep vein thrombosis

RFLP: Restriction fragment length polymorphism

RNA: Ribonucleic acid

SNP: Single nucleotide polymorphism

CNV: Copy number variation

INR: International normalized ratio

PT: Prothrombin time

PE: Pulmonary embolism

CHAPTER I

INTRODUCTION

Genes called *CYP2C9* and *VKORC1* are essential for the therapeutic response and metabolism of several drugs, especially anticoagulants like warfarin (Caldwell, M. D., Awad, T., & Johnson, J. A. 2008). Affected drug efficacy and potential adverse drug responses can result from genetic polymorphisms which influences medication utilization. For personalized medicine and drug therapy optimization, it is crucial to comprehend the effects of *CYP2C9* and *VKORC1* polymorphisms. (Johnson et al., 2017). An enzyme termed cytochrome P450 2C9, which is encoded by the gene *CYP2C9*, is predominantly involved in the metabolism of several medications, including warfarin, phenytoin, and nonsteroidal anti-inflammatory medicines (NSAIDs). Interindividual variations in drug metabolism and response can result from polymorphisms in the *CYP2C9* gene, which can impact the enzyme's function. *CYP2C92* (rs1799853) and *CYP2C93* (rs1057910) are the two most prevalent polymorphisms of *CYP2C9.* Compared to the *CYP2C9*1* allele of the wild type, these variations have lower enzyme activity (Haining et al., 2013). Vitamin K epoxide reductase, which is produced by the gene *VKORC1*, converts vitamin K epoxide into its active form as part of the vitamin K cycle. The anticoagulant medicine warfarin acts by blocking *VKORC1*, which lowers the amount of active vitamin K produced and hinders the blood clotting process. Warfarin helps to prevent coagulation of blood. This medication is commonly utilized to prevent or treat blood clots. It belongs to a class of drugs known as vitamin K antagonists (VKAs) and has been used extensively over many years. Warfarin is known for its effectiveness in reducing the risk of strokes, deep vein thrombosis (DVT), and pulmonary embolism (PE) (Ansell et al., 2008). The main purposes of warfarin are the prevention and treatment of diseases linked to irregular blood coagulation. Atrial fibrillation (AF) patients are frequently given warfarin to lower their risk of stroke, especially if they also have other risk factors. Blood clots in the deep veins and blood clots in the lung can both be prevented and treated with warfarin. It is frequently used in patients with a history of venous thromboembolism, especially post-replacement of the hip or knee procedures. A Patients with mechanical heart valves require anticoagulation therapy to prevent clotting on the valve. Warfarin is commonly used in

this population (Holbrook et al., 2012). Variations in warfarin dose requirements and response can be attributed to polymorphisms in *VKORC1*, which can modulate enzymatic activity. (Rieder et al., 2005). Patients' responses to and dosage needs for warfarin are affected by their *CYP2C9* and *VKORC1* polymorphisms. According to the theory, *VKORC1* and *CYP2C9* genetic variation are crucial in estimating the manner in which a sufferer might react to the anticoagulant medicine warfarin and the amount of it they will need to take. The relationship between genetic polymorphisms in *CYP2C9* and *VKORC1* and warfarin response was examined in many research. A study by Wadelius et al. (2007) found a significant relationship between *CYP2C9* variations and warfarin dosage needs. They discovered that specific *CYP2C9* polymorphisms, including *CYP2C92* and *CYP2C93*, were linked to decreased enzyme activity, which resulted in a slower rate of warfarin metabolism and increased susceptibility to the medication (Wadelius et al., 2007). Takeuchi identified *VKORC1, CYP2C9*, and *CYP4F2* as important genetic predictors of warfarin dose requirements in different genome-wide interaction study. Variations in the gene known as *VKORC1* are known to have an impact on target protein of the blood thinner warfarin's enzymatic activity. The research supported the hypothesis that particular *VKORC1* polymorphisms were linked to lower warfarin doses (Takeuchi et al., S 2009). Understanding the genetic variations that can affect the metabolism and response to particular drugs, particularly those that are metabolized by the CYP2C9 enzyme and those that target the vitamin K epoxide reductase complex 1 *(VKORC1),* is the goal of studies on *CYP2C9* and *VKORC1* polymorphisms in various population. By studying *CYP2C9* and *VKORC1* polymorphisms in different populations, researchers aim to identify patterns of genetic variation that may explain why certain individuals require higher or lower doses of warfarin to achieve the desired therapeutic effect. (Chen et al., 2009). To comprehend the racial and geographic variations in medication metabolism and response, it is crucial to examine the distribution and frequency of *CYP2C9* and *VKORC1* polymorphisms among various ethnicities. Certain polymorphisms may be more prevalent in some groups, which may have an effect on how well *CYP2C9*- and *VKORC1*-interacting medications work (Scott et al., 2013). To help with drug selection and dosage, pharmacogenetic testing for *CYP2C9* and *VKORC1* polymorphisms is being developed and utilize for scientific purposes. This can assist medical professionals in making

knowledgeable choices regarding the administration of medications like warfarin that are processed by *CYP2C9* or interact with *VKORC1* (Bruno et al., 2013). A pertinent scientific datum about the genetic variations that exist in this population will be revealed by the study about the allele frequencies and genotype distribution of *CYP2C9* and *VKORC1* polymorphisms in the Turkish Cypriot community especially in relation to drug metabolism and response to anticoagulant therapy. This knowledge can assist in the development of personalized medicine approaches that take into account the individualized genetic profiles of patients to improve treatment outcomes. Comprehensive investigations identifying the allele frequencies of *CYP2C9* and *VKORC1* polymorphisms in various communities around the world are required to fill one research gap. The majority of studies conducted to far have concentrated on particular ethnic groups including Caucasians, Africans, and Asians. There are several communities with distinctive genetic histories, though, that have not been thoroughly studied. A more accurate evaluation of drug response and dosage suggestions would be possible with an understanding of the allele frequencies in these populations (Hirsh et al., 2003). Although the medical importance of *CYP2C9* and *VKORC1* polymorphisms has been established for certain drugs, such as warfarin, there is a need for further research to evaluate the effect of polymorphisms on drug reaction across a broader range of medications. Investigating the association between *CYP2C9* and *VKORC1* genotypes and therapeutic outcomes in different populations would help identify potential variations in drug response and guide personalized treatment approaches (Zhang et al., 2011). The majority of research has been on *CYP2C9* and *VKORC1* common variants, and little is known about how rare variants affect medication response. Examining the incidence and functional effects of uncommon variations in various groups may assist to improve clinical outcomes and pharmacogenetic dosing algorithms (Schwarz et al., 2008). Numerous significant discoveries on medical importance of *CYP2C9* and *VKORC1* polymorphisms, personalized medicine, and anticoagulant therapy optimization have resulted from this research. Reduced enzyme activity and poorer warfarin metabolism have consistently been linked to the existence of the *CYP2C9* variant alleles (*2 and *3). This discovery prompted the creation of pharmacogenetic dosing algorithms that optimize warfarin dosage for specific patients by accounting for *CYP2C9* genotype (Johnson et al., 2011). Warfarin dosage needs have been

found to be significantly predicted by the *VKORC1 -1639G>*A variation. Lower doses of warfarin are needed to have the optimal anticoagulant effect in people who carry the variant allele. The accuracy of the initial warfarin dose prediction has showed promise when *VKORC1* genotype data is incorporated into dosing algorithms (Consortium et al., 2009). Genotypes of *CYP2C9* and *VKORC1* are connected to increase chance of bleeding incidents in those on warfarin. Clinicians can anticipate bleeding risk by identifying patients with *CYP2C9* variant alleles and the *VKORC1 -1639G>*A mutation, and then modify warfarin doses accordingly (Pir mohamed et al., 2013). Warfarin can now be used more effectively and safely thanks to knowledge of the *CYP2C9* and *VKORC1* genotypes. It has been demonstrated that adjusting warfarin dosages in accordance with unique genetic profiles increase the amount of duration spend on treatment, lowers the danger of over- or under-anticoagulation, and enhances patient outcomes (Anderson et al., 2007). In order to discover specific genetic variants, genotyping techniques are commonly utilized in studies of *CYP2C9* and *VKORC1* polymorphisms in various populations. Samples of blood or buccal swabs are taken from people from various populations. In order to carry out the study according to the ethical guidelines, informed consent and ethics approval are acquired (Shahin et al., 2011).

Studying the frequency of *CYP2C9* and *VKORC1* polymorphisms in the Turkish Cypriot population can advance pharmacological therapy methods using personalized medicine. Healthcare professionals can improve pharmaceutical dosing techniques by better understanding the distribution of these genetic variations, especially for medications like warfarin that are processed by *CYP2C9* and targeted by *VKORC1*. This may result in greater therapeutic results, fewer negative side effects, and better general patient care. The effectiveness of medications, particularly warfarin, can be severely impacted by variations in the *CYP2C9* and *VKORC1* genes. The study of these polymorphisms in the Turkish Cypriot community can help researchers pinpoint people who could be more susceptible to side effects or inadequate responses to therapy. It will be possible to gain insights and understanding of the Turkish Cypriot people by conducting research in that group. It offers details on this population's distinctive genetic makeup and traits, which may be different from those of other ethnic groups. Such knowledge can help us understand genetic variation more thoroughly and it will help us to modify medical procedures to better meet

the demands of the Turkish Cypriot community. An essential aspect to consider is the economic impact of healthcare. Researchers can evaluate the potential cost-effectiveness of genetic testing and personalized medicine strategies by examining *CYP2C9* and *VKORC1* polymorphisms in the different communities around the world. The judicious deployment of healthcare resources and the enhancement of cost-effectiveness in treatment strategies can both be accomplished by employing this knowledge to inform healthcare policy decisions and allocate resources efficiently. Investigations into the polymorphisms of *CYP2C9* and *VKORC1* within the Turkish Cypriot community have the potential to foster collaboration and facilitate knowledge exchange with researchers and experts in the field of pharmacogenetics on a global scale. This information sharing and teamwork can promote scientific cooperation, progress the subject, and deepen our understanding of pharmacogenetic variances worldwide. By studying *CYP2C9* and *VKORC1* polymorphisms within the Turkish Cypriot community, researchers can notably advance personalized medicine, bolster drug safety, and enhance the overall well-being of individuals within this specific demographic. Results of determining the genotype distribution and allele frequency of *CYP2C9* and *VKORC1* polymorphism study may have direct applications for patients, healthcare practitioners, and decision-makers, resulting in better healthcare outcomes and more efficient use of healthcare resources.

1.1 HUMAN GENOME ORGANIZATION:

The arrangement and structure of the DNA sequences that make up a human being's full genetic make-up are referred to as the human genome's organization. Gene organization, regulatory components, non-coding regions, repetitive sequences, and other genomic traits are all included in human genome organization. Understanding the physical configuration, functional connections, and regulatory systems that control gene expression and genomic interactions are all part of the study of genome organization (international Human Genome 2004). The entirety of a human's genetic code may be found in their DNA, which is known as their genome. It includes all the guidelines required to create and maintain a live entity. Genes, which encode proteins and RNA molecules that perform a variety of biological tasks, are discrete components of the human genome that are arranged in separate groups. The human genome contains about 3 billion base pairs of

DNAS, according to the Human Genome Project. Even while this quantity is astonishingly vast, it just makeup a minor percentage of the entire genome. Surprisingly, fewer than 2% of the genome is made up of genes that code for proteins. The remainder is composed of non-coding DNA, which was long referred regarded as "junk DNA" but is now understood to play crucial regulatory and structural roles. There are 23 sets of chromosomes in the human genome. Genes, which are DNA segments that hold the instructions for constructing proteins and other substances necessary for cellular functions, are found on these chromosomes. Exons, which are coding sections, and introns, which are non-coding regions, make up each gene. To create a mature RNA (Ribonucleic acid) molecule, exons and introns are joined together during transcription and splicing. In addition to genes that make proteins, the human genome also has a number of non-coding components that are essential for the structure and control of genes. These components include regulatory sequences that regulate where and when genes are expressed, such as enhancers and promoters. Long non-coding RNAs and microRNAs are two types of non-coding RNAs that participate in the regulation of gene expression and other cellular functions. The human genome is not static or linear in its arrangement. Distal sections of DNA can physically interact with one another to control gene expression because of its three-dimensional folding. By affecting the accessibility of DNA to cellular machinery, epigenetic alterations like DNA methylation and histone modifications also assist in the regulation of gene activity. In order to identify the genetic causes of human features, diseases, and the development of tailored therapy, it is essential to comprehend how the human genome is structured. The complexity of the human genome is being further understood through ongoing study, such as the discovery of genetic variations linked to specific diseases and the investigation of the gene-environment nexus. The complexity of the human genome is still being better understood via ongoing study, which also identifies genetic variations linked to diseases and investigates the interplay between genes and the environment (Lander et al., 2001). The entire collection of DNA sequences that make up a human being's genetic material is referred to as their genome. The human genome can be split into distinct organizational levels, starting with the smallest unit of DNA and ending with the largest unit of chromosome. DNA, also referred to as deoxyribonucleic acid, is a molecule that contains the genetic information required in order to build up,

operation, and reproduce of every known living thing. DNA is used to transmit genomic material from parents to offspring. For the transmission and preservation of genetic information, it is crucial. Nucleotides make up DNA, a lengthy, double-stranded molecule. Adenine (A), thymine (T), cytosine (C), and guanine (G) are the four nitrogenous bases that make up each nucleotide. Each nucleotide also contains a phosphate group and a sugar molecule (deoxyribose). By creating hydrogen bonds between complimentary bases, the two DNA strands are kept together. The distinctive double helix structure is created by the pairing of adenine with thymine and cytosine with guanine. James Watson and Francis Crick are credited with discovering the structure of DNA in 1953. They put forth the double helix concept, which completely altered how we think about DNA and how it affects genetic heredity. The accurate replication of DNA during cell division made possible by the double helix structure ensures the reliable transmission of genetic information The DNA molecule serves as the repository of the genetic code responsible for determining the sequential arrangement of amino acids within proteins. Proteins are essential for many biological functions and serve as the basis of cells. Protein synthesis, also known as gene expression, is the process by which the nucleotide sequence in DNA determines the amino acid sequence in a protein. This procedure includes transcription, which turns DNA into messenger RNA (mRNA), and translation, which uses mRNA as a blueprint to create proteins. The entire set of genetic data in a human individual, or the human genome, is encoded in DNA. There are 23 pairs of chromosomes in the human genome, totaling about 3 billion base pairs. It has between 20,000 and 25,000 protein-coding genes as well as non-coding sections that are crucial for the organization of the genome and the control of genes (Watson, J. D., & Crick, F. H. 1953). The process by which cells create precise replicas of their DNA is known as DNA replication. It is an essential procedure that guarantees the precise transmission of genomic information from one cell generation to the following during cell division. The two strands of DNA that are coiled around each other have to unfold to be able to duplicate, the two DNA strands must separate to form 2 new complementary strands using the original DNA template (Alberts et al., 2002).

Genes are DNA (deoxyribonucleic acid) segments that act as the fundamental functional components of heredity. They contain the information or code required to produce

particular proteins or functional RNA molecules, which are essential for the control, structure, and function of cells and organisms. Within a cell's nucleus, chromosomes contain genes. Through a process known as gene expression, each gene carries the instructions necessary to create a certain product, such as a protein or RNA molecule. DNA is translated into RNA during gene expression, and RNA is then translated into a protein. Physical qualities, physiological functions, and disease susceptibility are only a few of the traits and characteristics that are determined by genes in living things. They contribute to the continuity and variety of life since they can be inherited from parents and passed on to offspring. The study of genes, their structure, function, and the processes behind their transmission and expression are at the heart of genetics. Understanding the genetic foundation of traits, diseases, and this information enables the interaction between genes and the environment. Although not all genes code for proteins, they are frequently linked to non-protein coding regions. Certain genes yield functional RNA (Ribonucleic acid) molecules essential for processes such as protein synthesis and gene regulation. (Lewin et al., 2007).

DNA (deoxyribonucleic acid) and proteins found in the cell nucleus make up the structures known as chromosomes. They play a pivotal role in the transmission and functionality of living organisms by conveying the genetic information that is passed from one generation to the next, from parents to offspring. When chromosomes compress and coil tightly during cell division, they become apparent as thread-like filaments. They are made up of lengthy DNA molecules that are encircled by histone-like proteins. Several sequential folding stages contribute to the subsequent organization of this DNA-protein complex, leading to its compact conformation. Genes, which are particular DNA segments that encode directions for creating proteins and necessary for the health and growth of an organism, are found in chromosomes. Each gene contains the instructions needed to create a certain protein or carry out a particular task within the cell. In the majority of their bodily cells, humans normally have 46 chromosomes in total, with the exception of their reproductive cells (sperm and eggs), which have 23 chromosomes. The 23rd pair of chromosomes, often known as the sex chromosomes, determines a person's sex, while the first 22 pairs are referred to as autosomes. In the realm of human chromosomal sex determination, males are characterized by the presence of one X chromosome and one Y

chromosome (XY), while females are distinguished by the possession of two X chromosomes (XX). Chromosomes are essential for the precise distribution of genetic material to daughter cells during cell division. The two primary processes of cell division are meiosis and mitosis. Somatic cells divide during the mitotic phase to create two genetically analogous offspring cells. Meiosis is a specific type of cell division that takes place in reproductive cells and produces gametes (sperm and eggs). One key feature of meiosis is that it reduces the number of chromosomes in these cells by half compared to regular body cells. This reduction occurs through two stages: meiosis I and meiosis II. Through the process of reducing the chromosome count during gametogenesis, the ensuing zygote formed upon the fusion of gametes during fertilization attains the requisite and balanced chromosomal complement. Chromosome structure is dynamic and varies throughout the various stages of the cell cycle. Chromosomes are made up of two sister chromatids, which are genetically identical copies of each chromosome, prior to cell division. The centromere, a protein complex, holds these sister chromatids together. The sister chromatids split apart and are dispersed to the daughter cells during cell division (Alberts et al., 2002)

Human genome also contains non-coding regions. On-coding areas are sections of DNA that lack protein-coding genes, also referred to as non-coding DNA or non-coding sequences. Interestingly it is believed that these regions were functional, these sections were frequently called as “junk DNA.” Whereas, research in recent years has shown that non-coding regions have crucial regulatory roles in a variety of cellular processes. Non-coding regions can be further classified into two different categories. In genes, there are segments called exons that contain coding information, and these exons are separated by non-coding segments known as introns. The introns are found in the pre-mRNA transcription process however they are not found in mature mRNA. RNA splicing, on the other hand, involves the removal of introns and the joining of exons to create mature messenger RNA (mRNA), which can then be translated into proteins. Research has revealed that introns, traditionally thought of as “junk DNA” really serve significant regulatory functions in the expression of genes, alternative splicing, and the emergence of new genes (Mattick et al., 2006). Intergenic regions are segments of non-coding DNA that are located between genes. They comprise a large percentage of the genome and were

once thought to be functionless. Latest studies, demonstrate that intergenic areas contain a variety of regulatory components that regulate the expression of nearby genes, including promoters, enhancers, and silencers. These areas are essential for effective gene regulation and development because they can interact with genes over vast distances to affect their transcriptional activity (Shlyueva et al., 2014).

1.1.1 HUMAN VARIATION AND POPULATION:

Human diversity encompasses the range of genetic, phenotypic, and cultural variances observable among human populations and individuals. These variances are the outcome of intricate interplay social, environmental, and genetic factors. It is essential to comprehend human variety in order to advance sciences like genetics, anthropology, medicine, and public health. Genetic variety describes variations in DNA sequences between individuals. These variants can take many different forms, from small structural differences like insertions, deletions, and duplications to larger genetic diversity at the level of single nucleotide alterations (SNPs). Natural selection, recombination, mutation, and other processes all contribute to genetic variety. It is in charge of the physical diversity, disease susceptibility, and treatment responsiveness in human populations (Novembre et al., 2018). Human variety is influenced by both genetic and environmental influences. Individuals' and populations' physical attributes can be influenced by environmental factors like climate, food, and cultural customs. Populations inhabiting diverse temperature environments may undergo adaptations in characteristics such as skin pigmentation, somatic morphology, and metabolic physiology in order to better suit their specific ecological contexts. Population genetic adaptations and variances can also be influenced by diet and cultural activities like farming or pastoralism (Jablonski et al., 2014). Based on genetic, linguistic, or geographic factors, human populations can be divided into a variety of groups these categories are not rigid and distinct. Instead, they represent a range or continuum of genetic diversity. In other words, there is a lot of overlap and shared genetic traits among different populations, and it's often not accurate to think of them as completely separate or isolated groups. This highlights the complexity and interconnectedness of human diversity. By identifying patterns of genetic variation and ancestry, methods like genetic clustering and principal component analysis can be used to evaluate the structure of human populations (Hancock et al., 2008). It is crucial to

remember that race is now universally regarded as a social construct with no biological basis. Historically, race was used to divide human populations into distinct groupings based on physical traits. It is challenging to create distinct racial groupings based only on genetic information since genetic research have demonstrate that the genomic variety within community is far greater than the variation between populations (Rosenberg et al., 2002). Studies examining human genetic variation have provided insights into our evolutionary history, migration patterns, and adaptation to different environments. These studies utilize various methods, including genetic sequencing, genome-wide association studies, and analysis of ancient DNA. They have helped shed light on the genetic basis of certain diseases, drug responses, and other phenotypic traits (Lachance et al., 2018).

1.1.2 IMPORTANCE OF ALLELE FREQUENCIES AND GENOTYPE DISTRIBUTION AMOUNG POPULATION:

Understanding the genetic composition of populations and the long-term evolution of genetic mutation requires knowledge of allele frequencies and genotype distributions. Genetic risk factors for diseases can be discerned through the comparative analysis of allele frequencies and genotype distributions across populations, thereby elucidating discernible patterns of genetic variation and the impact of genetic drift. (Henn et al., 2012). The relative frequency of various gene alleles within a population is referred to as allele frequency. The collective occurrence of diverse genotypes or allele combinations within a population is designated as its genotype distribution. Numerous variables, like as mutation, genetic drift, migration, and natural selection, can affect the frequency of a specific allele or genotype (Jablonski et al., 2000). Comprehending these patterns can aid healthcare practitioners in formulating targeted interventions and screening initiatives, including the consideration that specific genetic variations may exhibit higher prevalence within particular population cohorts. (Norton et al., 2007). Additionally, variations in genotype distributions and allele frequencies might shed light on how populations have evolved over time. For instance, some genetic variations might be more prevalent in groups that have recently migrated or experienced genetic isolation, whereas others genetic variation might be linked to environmental adaptability (Khoury et al., 2006). In general, it is crucial to comprehend genotype distributions and allele frequencies for a

variety of disciplines, including anthropology, evolutionary biology, and medicine. Researchers can learn more about the biological and cultural influences that have molded human populations over time by examining patterns of genetic variation. Understanding genetic diversity, evolution, disease susceptibility, and population health depend greatly on the study of allele frequency and genotype distribution among populations (Hindorff et al., 2018). The significance of genotype distribution and allele frequency is highlighted in the following main points.

1.1.2.1 Diversity in Genetics and Evolution:

The percentage of a given allele within a population is referred to as allele frequency. Researchers can evaluate the genetic diversity within and between groups by looking at allele frequencies Genetic diversity is indispensable for a species to undergo evolutionary adaptation and acclimatize to shifting environmental conditions. Allele frequency patterns can be studied to learn more about population genetic linkages, migration patterns, and evolutionary history (Relling et al., 2015).

1.1.2.2 Pharmacogenetics and Disease Susceptibility:

Genetic variations can affect a person's susceptibility to diseases. The allelic frequency of disease-associated variants within a population can elucidate valuable insights regarding the prevalence and geographical distribution of specific illnesses. Knowing the frequency of alleles can assist identify groups more likely to have a certain genetic condition and can direct specialized preventative and intervention methods. Pharmacogenetics studies how a person's response to medication is influenced by genetic differences. The efficacy and safety characteristics of medications can be affected by variations in allele frequencies between populations (Lewontin et al., 1972).

1.1.2.3 Population Health and Public Health Genetics

The comprehension of disparities in population health and the refinement of healthcare strategies are facilitated through the application of genotyping and the analysis of allele distribution within the domains of population health and public health. Public health initiatives, genetic counseling, and screening programs can benefit from genetic data collected at the population level. It supports the discovery of population-specific genetic risk factors and the creation of individualized treatment plans (Nei et al., 1987).

1.1.3 THE EFFECT OF HUMAN POLYMORPHISM IN DISEASE:

Human polymorphisms, which are differences in each person's DNA sequence, can have a big impact on how a disease develops, how quickly it spreads, and how well a therapy works. The function of proteins, levels of gene expression, and immunological response are just a few of the components of disease biology that polymorphisms can affect. They can occur in both coding and non-coding sections of the genome. Human polymorphisms can exert significant effects on disease susceptibility through various mechanisms. Genetic predisposition, for instance, refers to an individual's heightened vulnerability to developing specific diseases, which may be augmented by the presence of certain single nucleotide polymorphisms (SNPs). For instance, particular genetic variations in genes linked to neurological illnesses, autoimmune disorders, cardiovascular disease, and cancer have been identified as risk factors. These polymorphisms may change how proteins function, obstruct regulatory processes, or impair the body's capacity to react to external stimuli (Visscher et al., 2012). Polymorphisms can influence the severity and progression of diseases. Variations in genes involved in inflammation, immune response, or cellular processes can affect disease outcomes. For instance, certain polymorphisms in the *CFTR* gene are associated with the severity of cystic fibrosis symptoms, while variants in the *APOE* gene are linked to the progression of Alzheimer's disease (Relling et al., 2015). Polymorphisms can impact how individuals respond to medications. (Visscher et al., 2017). Numerous uncommon genetic illnesses are caused by certain polymorphisms that impair normal gene function. These mutations may result in cellular dysfunction, structural defects, or enzymatic deficits. Examples include Huntington's illness, sickle cell anemia, and cystic fibrosis. For the sake of diagnosis, genetic counseling, and prospective therapeutic approaches, it is critical to comprehend the precise polymorphisms underlying these conditions. For instance, a variation in the lactase gene (LCT), which affects the capacity to digest lactose, is thought to be responsible for the high prevalence of lactose intolerance in some ethnic groups (Cirulli et al., 2010).

There are several types of polymorphisms in the human genome. Here are some commonly studied types.

1.1.3.1 SINGLE NUCLEOTIDE POLYMORPHISM (SNP):

The most prevalent type of polymorphism is known as single nucleotide polymorphism (SNP), which is defined by a single base pair difference at a particular location in the genome. SNPs can appear in the regulatory, non-coding, or coding sections of genes. They influence the functioning of genes, illness susceptibility, and protein function. For categorizing and researching SNPs, a comprehensive resource is the Single Nucleotide Polymorphism Database (dbSNP) (Sherry et al., 2001).

1.1.3.2 INSERTION/DELETION POLYMORPHISM (INDEL):

Indels are polymorphisms that have one or more nucleotides in the DNA sequence either inserted or deleted. Indels can result in frame-shift mutations, which change the gene's reading frame and impact how proteins function. Additionally, the dNTPS has details on indel polymorphisms (Sherry et al., 2001).

1.1.3.3 COPY NUMBER VARIATION (CNV):

CNVs are polymorphisms that affect how many copies of a specific DNA region are present. These variations may contain duplications, deletions, or intricate rearrangements and can range in size from kilobases to megabases. Gene dosage, gene structure, and disease susceptibility can all be affected by CNVs. CNVs can be listed in the Database of Genomic Variants (DGV), which is a useful tool (MacDonald et al., 2014).

1.1.3.4 TANDEM REPEAT POLYMORPHISM:

Tandem repeats represent DNA sequences characterized by the recurrent replication of a specific pattern of nucleotides. Variations in the number of repeats are a result of polymorphisms in tandem repeats. Tandem repeat polymorphisms are frequently utilized in genetic studies for gene mapping, population genetics, and forensic applications. They are likewise known as microsatellites or short tandem repeats (STRs) (Buschiazzo et al., 2006).

1.1.3.5 STRUCTURAL VARIANTS:

Larger-scale genomic changes, including as insertions, deletions, inversions, and translocations, affecting segments of DNA with lengths ranging from several hundred base pairs to megabases, are referred to as structural variations. These mutations can exert

substantial influence on the structure, functionality, and susceptibility to diseases of genes. Resources like the Database of Genomic Structural Variation (dbVar) give information on these variants. Comprehensive cataloging and characterization of structural variants are ongoing work (MacDonald et al., 2014).

1.2 THE DISCOVERY OF WARFARINS:

An anticoagulant drug called warfarin utilize to prevent blood clots from growing or developing in blood arteries, which can cause life-threatening conditions like deep vein thrombosis (DVT), pulmonary embolism, and stroke. Warfarin functions as an anticoagulant by perturbing the physiological hemostatic mechanism, thereby attenuating the propensity for blood coagulation and clot formation (Krawczak et al., 1998). Individuals afflicted with medical conditions predisposing them to an elevated risk of thrombotic events, including atrial fibrillation (characterized by irregular heart rhythm), heart valve replacement, or a prior history of venous thromboembolic events, are commonly subjected to warfarin therapy. Furthermore, warfarin is administered to patients who have undergone surgical procedures or who are expected to undergo prolonged periods of immobility with the objective of prophylactically mitigating the propensity for venous thrombosis. (Mills et al., 2011). Warfarin is offered as a tablet, and it is typically taken once day at the same time each day. Depending on the results of routine blood tests, the dose of warfarin may need to be changed to keep the blood thin enough to avoid clots but not so thin as to raise the risk of bleeding. Adherence to a prescribed dietary regimen and the diligent disclosure of concurrent medication usage to healthcare practitioners are imperative due to the potential interactions of warfarin with both dietary constituents and other pharmaceutical agents (Hirsh et al., 2004).

It’s a fascinating story how warfarin was discovered. Before its anticoagulant characteristics were found, warfarin was initially utilized as rat poison. Early in the 1950s, warfarin underwent its first human trials before becoming officially accepted for medical usage in 1954. Studies and experiments led to the development of warfarin, an anticoagulant drug that is now extensively used. Here is a summary of how warfarin was discovered. Farmers in the northern regions of the United States and Canada observed the emergence of bleeding diseases in cattle grazing on sweet clover during the 1920s.

Subsequent research revealed that sweet clover plants could occasionally become infected with a mold known as "coumarin mold" or "sweet clover disease." The root cause of this bleeding disorder was identified as coumarin, a naturally occurring substance found in sweet clover (Federer et al., 1929). In the early 1940s, the U.S. Army financed research aimed at developing an oral anticoagulant to prevent blood clotting in soldiers. American scientist Karl Link and his team at the University of Wisconsin sought to find a safer alternative to the anticoagulant dicumarol. The Link-led group synthesized several coumarin derivatives and conducted animal testing. Among these derivatives, 3,3'-Methylenebis(4-hydroxycoumarin) exhibited potential anticoagulant properties. In 1948, the Wisconsin Alumni Research Foundation (WARF) secured the first patent for the chemical 3,3'-methylenebis(4-hydroxycoumarin), which was subsequently named warfarin by combining "coumarin" and "WARF." Subsequently, other pharmaceutical companies, most notably DuPont and the Wisconsin Alumni Research Foundation, manufactured and marketed warfarin as a medication. Since its initial medical approval in 1954, warfarin has become one of the most frequently prescribed oral anticoagulant medications. Over time advancements in the management of warfarin therapy have been facilitated by the development of |Internalize normalize ratio (INR) system. This system allows doctor tailor warfarin dosage for individual patients based on their desired therapeutic target range, as INR offers a standardized measurement for blood clotting (Johnson et al., 2017)

1.2.1 THE VITAMIN K AND IT`S CYCLE

1.2.1.1 VITAMIN K:

Vitamin K is a class of fat-soluble vitamins that contribute an important role in blood clotting, bone metabolism, and other important bodily functions. There are two primary types of vitamin K: vitamin K1 (phylloquinone), which can be discovered in agricultural products, and vitamin K2 (menaquinone), which is synthesized by bacteria in the gut and also discovered in foods made with animals (Howell et al., 2010). Vitamin K1 is primarily used by the liver to activate clotting factors, while vitamin K2 is involved in regulating calcium metabolism in bones and soft tissues. Deficiency in vitamin K can result in excessive bleeding and heightened danger of bone fractures (Suttie et al., 2011). Good dietary sources of vitamin K include leafy green vegetables, such as kale, spinach, and

broccoli, as well as some animal-based foods like liver and egg yolks. In addition, vitamin K supplements are available in various forms, including tablets, capsules, and liquid drops. As with any supplement, it is crucial to consult medical professionals before beginning to take vitamin K supplements (Shearer et al., 2009).

1.2.1.2 USES OF VITAMIN K:

Vitamin K has several important uses in the body. Here are some of the main ones. Vitamin K performs a crucial part in blood clotting by permitting to activate clotting factors in the liver. Vitamin K2 plays a role in controlling the way that bones and soft tissues use calcium. For the maintenance of healthy bones and the prevention of osteoporosis, adequate vitamin K intake is required. Health for the heart: Research has indicated that vitamin K2 may lessen the risk of heart disease by preventing arterial calcification. Memory and cognitive performance in elderly persons have been shown to be enhanced by vitamin K. Health of the skin: Vitamin K may help with skin appearance, especially in fading the look of dark circles beneath the eyes (Theuwissen et al., 2014). While vitamin K may offer a range of potential health benefits, comprehensive research is imperative to attain a complete understanding of its effects and to unlock its full potential for the treatment or prevention of specific ailments. Before commencing the utilization of vitamin K supplements, it is advisable to consult with a healthcare professional, particularly if any medications are already being taken or if a pre-existing medical condition is present. (Theuwissen et al., 2014).

1.2.1.3 DEFICIENCY OF VITAMIN K:

Lack of vitamin K can be caused by a number of conditions, including a poor diet, malabsorption problems, liver disease, or prolonged antibiotic usage. A deficit in vitamin K can give rise to various health issues, among which is notably impaired blood coagulation. Vitamin K is necessary for the blood's clotting components to activate. In consideration of this circumstance, a deficiency in vitamin K may culminate in atypical bleeding manifestations, encompassing occurrences such as epistaxis (nosebleeds), gingival hemorrhage, or pronounced menorrhagia. Increased risk of fractures: Vitamin K, which aids in the activation of proteins that bind calcium to the bone matrix, is also crucial for the health of your bones (Friday et al., 1989). Particularly in older persons, a vitamin

K shortage might increase the risk of fractures. Low levels of vitamin K have been Associated with greater danger of cardiovascular disease, according to studies. Proteins that aid in preventing calcium accumulation in the arteries are activated by vitamin K. Moreover, impaired brain function: Vitamin K plays a crucial role in the formation and maintenance of brain cells by assisting in the activation of proteins. Vitamin K insufficiency can raise the risk of dementia and impair cognitive function. A lack of vitamin K may raise the chance of developing some cancers, including prostate, colon, and liver cancer. Vitamin K has been found to have anti-cancer properties. Overall, a vitamin K shortage can have negative effects on health, therefore it's critical to get enough of the vitamin through diet or supplements to keep your health at its best (Price et al., 1985).

1.2.1.4 EXCESS OF VITAMIN K:

Hypervitaminosis K, commonly known as an excess of vitamin K, is an uncommon condition that often only affects those who use large quantities of vitamin K supplements. A fat-soluble vitamin called vitamin K is crucial for bone metabolism and blood coagulation. Excessive intake of vitamin K can precipitate a spectrum of manifestations, notably including the diminished efficacy of anticoagulants such as warfarin, as vitamin K plays a pivotal role in promoting coagulation and, consequently, its surplus can attenuate the effectiveness of blood thinners. Jaundice can result from an excess of bilirubin in the blood, which can be brought on by hypervitaminosis K. Too much vitamin K can prevent the body from absorbing iron, which results in anemia. High doses of vitamin K can harm the liver, especially in those who already have liver disease. Vomiting and nausea can be brought on by too much vitamin K irritating the stomach lining (Relling et al., 2011).

1.2.1.5 STRUCTURE OF VITAMIN K:

Two naturally occurring forms of vitamin K, vitamin K1 (phylloquinone) and vitamin K2 (menaquinone), are included in the vitamin K family of fat-soluble vitamins. A naphthoquinone ring system with a side chain of variable length distinguishes the chemical makeup of vitamin K. While vitamin K2 (menaquinone) has a variable-length side chain with between 1 and 13 isoprene units, vitamin K1 (phylloquinone) has a phytl

side chain with four isoprene units. The function of vitamin K in the body is due to its naphthoquinone ring structure (Bookes 1999). As a cofactor for the enzyme gamma-glutamyl carboxylase, which adds carboxyl groups to particular amino acids in these proteins to activate them, vitamin K is necessary for the activation of some proteins involved in blood clotting and bone metabolism. Overall, vitamin K's distinct chemical structure is crucial to its function in controlling blood clotting and bone metabolism, and any changes to this structure may have an impact on the vitamin's performance in the body (Relling et al., 2011).

1.2.1.6 VITAMIN K CYCLE:

The complicated biochemical process known as the vitamin K cycle, also called the vitamin K-dependent gamma-carboxylation cycle, is essential for the post-translational modification of particular proteins involved in blood clotting, bone metabolism, and vascular health. The vitamin K cycle is described in depth. The term "vitamin K" describes a class of chemically related substances known as quinones. Vitamin K1 (phylloquinone), which is obtained from plant sources including leafy green vegetables, and vitamin K2 (menaquinone), which is produced by gut bacteria and also present in animal-based meals, are the two main types of vitamin K. Following consumption, dietary lipids and vitamin K are both absorbed in the small intestine (Friday et al.,1989). For effective absorption, bile salts and pancreatic enzymes are necessary. Chylomicrons, which are lipoprotein particles, are formed when vitamin K is ingested and carry it to the liver. Vitamin K undergoes a two-step activation process in the liver. The enzyme vitamin K epoxide reductase (VKOR) first transforms it into vitamin K hydroquinone (KH2). This phase is followed by the conversion of vitamin K epoxide (KO), a reducing agent, into vitamin K quinone (K). The enzyme gamma-glutamyl carboxylase (GGCX) then catalyzes the conversion of KH2 back to vitamin K epoxide, which is the active form of the compound (Norton et al., 2007). The energy needed for carboxylation is produced in this step. Gamma-glutamyl carboxylase (GGCX) uses vitamin K epoxide, the activated form of vitamin K, as a cofactor. A carboxyl group is added to particular glutamate residues on target proteins during gamma-carboxylation. The functional activation of vitamin K-dependent proteins (VKDPs) depends on this process (The biological action of VKDPs depends on carboxylation. Blood clotting agents such prothrombin, Factors VII, IX, and

X are some of the most well-known VKDPs. These clotting factors can attach to calcium ions thanks to carboxylation, which facilitates the development of blood clots. Other VKDPs include proteins associated to vascular health, including matrix Gla protein (MGP), and proteins involved in bone metabolism, like osteocalcin. The enzyme VKOR converts vitamin K epoxide to vitamin K hydroquinone (KH2), which is the active form of vitamin K after gamma-carboxylation. The continual availability of active vitamin K for ongoing carboxylation processes depends on this activity (Norton et al., 2007).

1.2.2 WARFARINS THERAPY:

In order to stop blood clots from developing or growing larger, a treatment called warfarin therapy uses the drug warfarin. Blood clots can lead to major health issues such pulmonary embolism, heart attack, and stroke. An anticoagulant drug called warfarin prevents the blood from clotting by preventing certain clotting components from being produced. The dosage is normally changed based on the outcomes of routine blood tests that gauge the blood's clotting time. It is typically given orally in tablet form. Deep vein thrombosis, pulmonary embolism, and atrial fibrillation are all frequently treated with warfarin medication. People who have had their heart valves replaced or who are at a high risk of getting blood clots may also be prescribed it. It is significant to highlight that a healthcare professional must closely monitor and manage warfarin therapy. This is due to the medication's potential for major adverse effects, like bleeding, and the need to carefully regulate dosage to strike a balance between preventing blood clots and reducing consequences from bleeding. In addition to modifying their food and avoiding certain activities that could raise their risk of bleeding or injury, people taking warfarin therapy may also need to alter their way of life (Aithal et al., 2003).

1.2.2.1 HOW WARFARIN WORKS:

Warfarin prevents the blood from producing specific clotting factors, which is how it works. For the blood to clot effectively, these clotting factors—which include vitamin K-dependent factors must be present. Warfarin slows down the blood clotting process and lowers the chance of blood clots forming or growing larger by preventing their creation. Warfarin accomplishes this by inhibiting the activity of a vitamin K epoxide reductase enzyme (Huang et al., 2013). The generation of clotting factors requires the conversion of

vitamin K epoxide to its active form, which is carried out by this enzyme. Warfarin inhibits the production of blood clotting factors that rely on vitamin K by blocking the activity of the enzyme responsible for their synthesis. It is of paramount importance to grasp the mechanism of action of warfarin, which does not involve the dissolution of pre-existing thrombi but rather functions to impede the augmentation of extant blood clots or the genesis of novel ones. Consequently, the attainment of the full therapeutic effect of warfarin may necessitate a protracted timeframe spanning several days to weeks. Based on routine blood tests that determine the blood's clotting time, the dosage of warfarin is carefully regulated. The objective is to maintain equilibrium between preventing blood clots and reducing complications from bleeding, which could be a side effect of warfarin medication (Sherry et al., 2001).

1.2.2.2 DOSAGE AND ADMINISTRATION OF WARFARINS:

Warfarin dose and administration differ based on the patient's age, weight, and medical history, among other variables. Regular blood tests that gauge the blood's clotting time are frequently used to alter the dosage. The objective is to keep the International Normalized Ratio (INR) within the healthcare provider-set target range. The following chart provides an example of the dosage and target INR range for warfarin therapy (Haining et al., 2013).

Table 1: Dosage and Target INR Range for Warfarin

INDICATION	**INITIAL DOSE**	**TARGET INR RANGE**
Atrial fibrillation	2-5 mg once daily	2.0-3.0
Deep vein thrombosis or pulmonary embolism	5-10 mg once daily for first 2 days, then adjust based on INR	2.0-3.0
Heart valve replacement	2.5-10 mg once daily	2.5-3.5
Stroke prevention in high-risk patients	2-5 mg once daily	2.0-3.0

It is imperative to acknowledge that the prescribed dosage regimen and the specified target International Normalized Ratio (INR) range may necessitate modification contingent upon the individual patient's response to the medication and their inherent susceptibility to hemorrhagic complications. Vigilant adherence to medical directives and the consistent scheduling of periodic hematological assessments are of paramount importance in monitoring the efficacy and safety of warfarin therapy. (Haining et al., 2013).

1.2.2.3 MONITORING WARFARIN THERAPY:

For warfarin medication to be successful and safe, monitoring is crucial. The objective of this study is to maintain the International Normalized Ratio (INR) within the target range established by the healthcare provider. The right dose of warfarin is chosen based on the INR, a measurement of the blood's capacity to clot. The individual's medical status and the stability of their INR determine how frequently they are monitored. When starting warfarin therapy, patients typically need more frequent monitoring until their INR stabilizes within the target range. The frequency of monitoring can be decreased if the INR has stabilized. Based on the patient's health and other variables, the healthcare provider will choose the target INR range. Based on the person's response to the drug and their risk of bleeding problems, the target range may change over time. Adherence to regular INR monitoring appointments and timely disclosure of any modifications to medication, dietary, or lifestyle variables, with potential INR implications, are imperatives in anticoagulation management. If the patient suffers bleeding issues or if the INR is beyond the desired range, the healthcare provider may change the warfarin dosage. To maintain the security and efficacy of warfarin medication, the healthcare provider may also monitor the patient's liver function, kidney function, and other blood tests in addition to INR monitoring. It's crucial to notify the healthcare practitioner right away of any bleeding symptoms, such as unusual bruising, bleeding gums, or blood in the urine or stool (Whitworth et al., 2012).

1.2.2.4 POTENTIAL SIDE EFFECTS OF WARFARINS:

Bleeding is the most frequent adverse reaction to warfarin medication. From minor bruising to potentially fatal bleeding in the brain, stomach, or other organs, this can occur. Rarely, taking warfarin can result in skin necrosis, or the death of skin tissue. This usually

happens in the first few days to weeks after beginning warfarin therapy and is more frequent in those who have a protein C deficit. Warfarin medication used for a long time has been linked to a higher risk of osteoporosis, a disorder in which the bones become brittle and fragile. Some people have had hair loss as a result of warfarin medication. Rarely, taking warfarin may result in allergic reactions like skin rashes, hives, or breathing difficulties. Added negative consequences The following side effects of warfarin medication are also possible: fever, nausea, vomiting, diarrhea, and stomach pain. Any adverse effects or symptomatic developments experienced during warfarin administration should be promptly communicated to a healthcare provider. To control adverse effects, the doctor may reduce the warfarin dosage or recommend additional drugs. Warfarin therapy may need to be stopped in specific circumstances (Keeling et al., 2011).

1.2.3 STEREOCHEMISTRY:

The oral anticoagulant medicine warfarin is a member of the vitamin K antagonists (VKAs) pharmacological class. It exerts its therapeutic effect by inhibiting the activity of vitamin K epoxide reductase (VKOR), thereby disrupting the normal functionality of vitamin K-dependent clotting factors (Bell et al., 2009).

1.2.3.1 ABSORPTION AND DIETARY SOURCES:

The small intestine is where vitamin K is mostly absorbed from food. It comes in two different forms: vitamin K2 (menaquinone), which is produced by gut bacteria and can also be found in some animal-based meals, and vitamin K1 (phylloquinone), which is found in green leafy vegetables (Booth et al., 1998).

1.2.3.2 ACTIVATION:

When vitamin K is absorbed, a process known as activation transforms it from its inactive form to its active form. The liver is the primary site of activation. Vitamin K epoxide reductase (VKOR), an enzyme, is needed for a sequence of events that turn vitamin K into its active form, vitamin K hydroquinone (KH2), as part of the activation process. The function of VKOR depends on vitamin K itself (Newman et al., 2008).

1.2.3.3 CARBOXYLATION OF PROTEINS:

The carboxylation of some proteins requires the active form of vitamin K, KH2. A carboxyl group is added to particular amino acids in the target proteins during the

carboxylation process. Gamma-glutamyl carboxylase (GGCX) is an enzyme that catalyzes the carboxylation process. Gamma-carboxyglutamate (Gla) residues, which are essential for the biological function of these proteins, are created as a result of this process from certain glutamate residues (Furie et al., 2004).

1.2.3.4 BLOOD CLOTTING:

The role that vitamin K plays in the coagulation of blood is one of its main activities. Certain proteins, including prothrombin, factors VII, IX, and X, as well as proteins C and S, are carboxylated in the liver, which enables them to attach to calcium ions and phospholipids on the outside of blood vessels. This binding starts the coagulation cascade, which causes blood clots to form to stop bleeding (Stenflo et al., 1991).

1.2.3.5 VITAMIN K EPOXIDE REGENERATION:

Vitamin K is carboxylated to produce vitamin K epoxide (KO), which must then be regenerated to produce vitamin K in its active state. The enzyme VKOR participates in a procedure known as the vitamin K epoxide reductase (VKOR) pathway, which is how regeneration happens. The activation, carboxylation, and regeneration processes that make up the vitamin K cycle enable vitamin K to be used repeatedly for a variety of purposes. It is crucial to remember that the vitamin K cycle is strictly controlled to keep the body's levels of active vitamin K at their ideal levels (Aumüller et al., 2020).

1.2.4 PHARMACOKINETICS & PHARMACODYNAMICS OF WARFARINS

1.2.4.1 PHARMACOKINETICS:

The study of a drug's absorption, distribution, metabolization, and elimination by the body is known as pharmacokinetics. It's crucial to comprehend warfarin's pharmacokinetics in order to utilize the medication safely and effectively. Warfarin is effectively absorbed after being administered orally. However, a variety of elements, including food, medications, and gastrointestinal conditions, can have an impact on a substance's bioavailability. In the plasma, warfarin is 99% protein-bound to albumin. Its distribution is therefore constrained to the plasma compartment and has a tiny volume. Warfarin undergoes substantial cytochrome P450 (CYP) enzyme system metabolism in the liver, particularly *CYP2C9*. The R-enantiomer of warfarin is metabolized more quickly than the S-enantiomer because the metabolism is stereoselective. This may lead to greater plasma

levels of the S-enantiomer, which is what gives warfarin its anticoagulant properties (Theuwissen et al., 1985). Warfarin is predominantly excreted in the urine and processed by the liver for elimination. Warfarin has an elimination half-life of 20 to 60 hours, depending on the patient's age, genetics, and liver function. Pharmacokinetics-affecting variables: A number of variables, including genetic variants in *CYP2C9* and vitamin K epoxide reductase (VKOR), medication interactions, age, gender, body weight, and liver function, can impact the pharmacokinetics of warfarin. Additionally, dietary vitamin K intake may modify the activity of VKOR, which may have an impact on the pharmacokinetics of warfarin. In conclusion, the well-known pharmacokinetics of warfarin are characterized by high protein binding, substantial liver metabolism, and stereoselective metabolism of its enantiomers. In the process of modifying a patient's therapeutic regimen and monitoring its safety and efficacy, it is imperative to possess a comprehensive understanding of the variables that impact the pharmacokinetics of warfarin (Huddart et al., 2012).

1.2.4.2 PHARMACODYNAMICS:

Pharmacodynamics is known as the connection between a drug's concentration and its physiological effects. The pharmacodynamics of warfarin include its anticoagulant activity, which is connected to its capacity to prevent the manufacture of coagulation factors II, VII, IX, and X that is dependent on vitamin K. By interfering with the vitamin K cycle, which is necessary for the production of clotting components in the liver, warfarin functions as an anticoagulant. The reduction of vitamin K epoxide to its active form, vitamin K, is catalyzed by the enzyme vitamin K epoxide reductase (VKOR). Due to warfarin's inhibition of VKOR, vitamin K levels are decreased and its availability for the production of clotting components is decreased. Warfarin takes around 5-7 days to establish steady-state anticoagulation and has a delayed onset of effect. Warfarin influences the production of clotting components with a long half-life, which can take several days to decrease, which explains why (Aiithal et al., 2003). The international normalized ratio (INR), which is a standardized measurement of the prothrombin time (PT), is used to track the anticoagulant effect of warfarin. The PT calculates how long it takes for blood to clot after tissue damage. The international sensitivity index (ISI) is used to compute the INR by dividing the patient's PT by the mean normal PT. The ideal INR

range for the majority of patients is 2.0–3.0, though this can change based on the patient's unique risk factors for bleeding and thrombosis. Numerous variables, including genetic polymorphisms in CYP2C9 and VKOR, drug interactions, age, gender, body weight, liver function, and vitamin K intake through diet, can influence the pharmacodynamics of warfarin. In contrast, variables that lower the INR, such as poor adherence to therapy or changes in liver function, might raise the risk of thrombosis. Variables that increase the INR, such as drug interactions or changes in dietary vitamin K consumption, can increase the risk of bleeding. In conclusion, the pharmacodynamics of warfarin involve its ability to suppress the formation of clotting components that is dependent on vitamin K, which results in its anticoagulant effect. The International Normalized Ratio (INR), subject to influence by a multitude of variables necessitating consideration during the tracking of individualized medication for safety and efficacy, serves as a pivotal metric for monitoring the anticoagulant effect. (Sherry et al., 2001).

1.2 WARFARINS MONITORING:

In order to make sure that a patient's blood clotting time is within a therapeutic range while taking warfarin, the patient's International Normalized Ratio (INR) is routinely measured. The INR compares the patient's blood clotting time to a normal reference range and is a standard measure of blood clotting time. The therapeutic INR range for people taking warfarin normally lies between 2.0 and 3.0, though this might change based on the patient's health and other variables. Regular INR monitoring is required to make sure the patient's blood clotting time stays within the therapeutic range because both high and low INR levels might increase the risk of blood clots and bleeding, respectively. INR monitoring is commonly done every 2-4 weeks during the start of treatment and subsequently every 4–12 weeks during maintenance therapy, depending on the patient's particular response to warfarin. However, if the patient has changes in their medication, nutrition, or other circumstances that could alter the INR, the frequency of monitoring might be increase. Patients using warfarin should have regular clinical examinations in addition to INR monitoring to look for any signs of bleeding or other side effects. Patients should be duly advised to promptly seek medical attention in the event of any manifestation of warning signs or symptoms indicative of bleeding. For the purpose of

ensuring effective monitoring and management of patients undergoing warfarin therapy, healthcare practitioners are required to engage in close and interactive patient interactions (Limdi et al., 2008).

1.3.1 PROTHROMBIN TIME (PT) & THE INTERNALIZED NORMALIZE RATIO (INR):

1.3.1.1 PROTHROMBIN TIME:

Laboratory test called the prothrombin time (PT) calculates how long it takes for blood to clot. It analyzes the coagulation cascade's extrinsic pathway, focusing mainly on factors II (prothrombin), VII, and X Factors II, VII, IX, and X, which are dependent on vitamin K for synthesis, are among the coagulation factors that are affected by the oral anticoagulant drug warfarin. To detect the anticoagulant impact of warfarin and alter the dosage in order to maintain the appropriate therapeutic range, PT testing is mostly used in the context of warfarin therapy (Ansell et al., 2008). Because of this, people receiving warfarin therapy often have longer PTs than those who are not receiving the drug. The patient's blood clotting time is measured in seconds, and the results are compared to a laboratory reference range to see if it falls within the normal range. Depending on the patient's unique condition and other considerations, the target PT range for people receiving warfarin medication varies. A therapeutic PT range is typically 1.5–2.5 times that of the reference range. For instance, if the goal therapeutic range for a patient receiving warfarin medication is 18-37.5 seconds and the laboratory reference range for PT is 12-15 seconds. It is significant to remember that the PT is not used to change the warfarin dose on its own. Instead, PT findings are standardized across various labs and test methodologies using the international normalized ratio (INR). For individuals receiving warfarin therapy, the goal INR range is normally 2.0 to 3.0, though this can change based on the patient's unique condition and other circumstances. To ensure that patients on warfarin therapy receive appropriate monitoring and management, healthcare professionals should collaborate closely with the patients (Ansell et al., 2008).

1.3.1.2 INTERNALIZED NORMALIZE RATIO (INR):

A laboratory test called the international normalized ratio (INR) is used to keep track of individuals receiving warfarin therapy. The anticoagulant drug warfarin works by

preventing the liver from producing specific clotting components. To provide uniform monitoring and result interpretation across various laboratories and test methodologies, the prothrombin time (PT) values are standardized using the INR. By dividing the patient's PT by the laboratory reference PT and multiplying the result by the international sensitivity index (ISI), which can vary depending on the specific laboratory and reagent employed. The following equation can be used to determine the INR:

INR = (patient's PT / laboratory reference PT) ^ ISI

Depending on the patient's unique condition and other considerations, the target INR range for patients receiving warfarin medication varies. The target range is often between 2.0 and 3.0. For some patients, however, such as those with mechanical heart valves or a history of blood clots, the target INR range may be higher or lower. Depending on the patient's age, general health, and other drugs they are taking, the target range and frequency of monitoring may also change. For patients taking warfarin therapy, regular INR monitoring is necessary to make sure they get the right dosage of the drug. Patients should have their INR examined as frequently as their doctor advises, usually every 4 to 12 weeks but maybe more or less frequently depending on the patient's specific requirements. The patient's warfarin dosage may need to be changed if the INR diverges from the desired range in order to maintain the proper level of anticoagulation.

The following table provides a general guide to interpreting PT and INR values for patients on warfarin therapy.

Table 2: Prothrombin time and Internalize Normalize Ratio (INR) for Patients on Warfarin.

PT (SECONDRY)	**INR**	**INTERPRETATION**
< 12.0	<1	Low risk of bleeding
12.0-15.0	1.0	Normal range
16.0-21.0	1.5	Low therapeutic range (risk of thrombosis is increased)
21.0-25.0	2.0	Therapeutic range for most indication

26.0-30.0	2.5	High therapeutic range (risk of bleeding is increased)
>30.0	>3	High risk of bleeding

Healthcare personnel must contact closely with the patients to ensure proper monitoring and management of those taking warfarin therapy. Patients should be advised to seek medical attention if any of the symptoms or warning signs associated with bleeding appear. By properly monitoring and managing patients using warfarin medication, healthcare practitioners can help minimize the risk of issues and improve patient outcomes (Ansell et al., 2008).

1.3.2 WARFARIN MECHANISM:

An oral anticoagulant drug called warfarin is frequently prescribed to prevent and treat blood clotting issues. It works by interfering with the normal production and operation of liver-based, vitamin K-dependent clotting factors. By preventing the activity of the enzyme vitamin K epoxide reductase (VKOR), warfarin prevents blood clotting. The carboxylation of particular glutamate residues in clotting factors II, VII, IX, and X requires the active form of vitamin K hydroquinone, which is reduced from vitamin K epoxide by VKOR. These clotting components must undergo carboxylation in order to function properly in the blood coagulation cascade. Warfarin reduces the creation of the functional versions of clotting factors by inhibiting VKOR, which stops the regeneration of active vitamin K. With uncarboxylated glutamate residues, the generation of clotting factors II, VII, IX, and X is thus decreased. These elements have a role in the development of a fibrin clot, which aids in stopping bleeding. Warfarin's effects on functional clotting factors result in an anticoagulant effect that aids in preventing blood clot formation. Warfarin dosage requires vigilant monitoring and precise adjustments since excessive anticoagulation may increase the risk of bleeding, whereas inadequate anticoagulation may fail to prevent clot formation (Wadelius et al., 2007).

1.4 PHARMACOGENETICS:

Pharmacogenetics is the branch of research that looks into how a person's genetic differences can affect how they react to drugs. It investigates the connection between

genetic elements and medication efficacy, safety, and metabolism. Pharmacogenetics strives to tailor pharmacological therapy by taking a person's genetic profile into account to optimize medicine choice, dose, and reduce the likelihood of negative side effects. Pharmacogenetics refers to the study of how genetic variations can impact an individual's response to drugs. It focuses on identifying specific genetic variations, such as single nucleotide polymorphisms (SNPs), insertions/deletions (indels), or gene duplications, that can affect drug metabolism, transport, and target interactions (Relling et al., 2015). Genetic differences in the cytochrome P450 enzymes (CYPs), which are involved in drug metabolism, can affect how quickly medications are processed and eliminated from the body. The efficacy and toxicity of medications can be affected by changes in enzyme activity caused by different alleles of these genes (Zanger et al., 2013). The sensitivity or responsiveness of these targets to drugs can be impacted by genetic differences in the genes encoding pharmacological targets or receptors. Changes in these genes' drug binding affinities or downstream signaling pathways can affect how well certain medications work therapeutically (André et al., 2013). Drug transporter gene variants can affect how medications are absorbed, distributed, and eliminated from the body. The therapeutic concentration and bioavailability of drugs may be impacted by these differences (ASCPT News. (2013). The results of pharmacogenetic testing can be used to determine drug selection, dose modifications, and patient outcomes. It necessitates cost-effectiveness analysis, healthcare professional education, and inclusion into clinical practice guidelines (Caudle et al., 2017).

1.4.1 PHARMACOGENETICS AND WARFARINS:

The field of pharmacogenetics investigates how a person's genetic make-up influence an individual's drug responsiveness. It looks into how genetic variants can impact a person's capacity to metabolize, react to, and tolerate particular medications. Pharmacogenetics has proven to be particularly useful in understanding the diversity in response to warfarin treatment. Warfarin is an anticoagulant drug that is frequently prescribed. Although warfarin is frequently used to stop and cure blood clots, determining the proper dosage can be difficult. Age, body weight, food, and genetic variations are just a few of the variables that affect an individual's ideal dose. Warfarin sensitivity and metabolism can be affected by genetic variations in certain genes, which can result in a range of responses

and potentially harmful effects. The gene *CYP2C9*, which codes for an enzyme that breaks down warfarin in the liver, is one of the most thoroughly researched genes involved in warfarin metabolism. The enzyme activity of *CYP2C9* is decreased by a number of genetic variants, including the *2 and *3 alleles. Lower warfarin doses may be necessary for those who carry these variations to obtain the intended anticoagulant effect and prevent bleeding problems (Caldwell et al., 2008). *VKORC1,* a gene that codes for the first subunit of the vitamin K epoxide reductase complex, is another crucial gene in the pharmacogenetics of warfarin. Warfarin targets this enzyme because it is a key component of the vitamin K cycle, which is necessary for blood clotting. The susceptibility of a person to warfarin can be affected by genetic variations in the *VKORC1* gene. While certain variations are linked to improved sensitivity and need lower doses of warfarin, whereas sone other variations are linked to impaired sensitivity and need larger dosages for therapeutic benefit. These genetic variants in *CYP2C9* and *VKORC1* can be found by pharmacogenetic testing, which enables doctors to adjust the dosage of warfarin for each patient. Healthcare professionals can reduce the probability of negative effects like bleeding or inadequate anticoagulation by taking hereditary variables into consideration. While Pharmacogenetic testing is utilized to personalize drug treatment by identifying genetic variations that affect an individual's response to medications. When recommending warfarin, other variables like clinical and environmental aspects are also taken into account (Rieder et al., 2005).

1.4.1.1 *CYP2C9* POLYMORPHISM:

The connection between *CYP2C9* genetic variations and anticoagulation-related outcomes during warfarin medication was examined in the research by Higashi et al 2002. In order to genotype patients for *CYP2C9* polymorphisms, the researchers examined a cohort of 297 warfarin-treated patients. They discovered that persons with the *CYP2C9 *2* or **3* alleles needed lower warfarin doses and were more likely to experience bleeding problems. The study offered proof of the clinical importance of *CYP2C9* polymorphism in figuring out how much warfarin is needed and the likelihood of side effects (Higashi et al., 2002). Aquilante et al., 2006 study looked at how *CYP2C9* variations and other genetic polymorphisms affect the amount of warfarin that needs to be taken. They genotyped 206 patients receiving stable warfarin medication and looked at how genotypes correlated with

the amount of warfarin needed. According to the study, *CYP2C9* polymorphisms had a substantial impact on warfarin dosage, with those with the *CYP2C9 *2* or *3* alleles needing less amounts. The results underlined how crucial it is to take into account *CYP2C9* genotype when administering warfarin medication (Aquilante et al., 2006). The impact of these mutations on warfarin dosage was assessed subsequent to the genotyping of 284 individuals for *CYP2C9* polymorphisms by the researchers. In the Chinese population, it was revealed that *CYP2C9* polymorphisms exerted a substantial influence on the requisite dosage of warfarin. The study emphasized the importance of considering genetic factors, specifically *CYP2C9* polymorphisms, when determining warfarin dosing regimens tailored to specific ethnic populations (Lee et al., 2009).

1.4.1.2 *VKORC1* POLYMORPHISM:

The vitamin K cycle, which is essential for the manufacture of clotting factors, is catalyzed by an enzyme that is encoded by the gene *VKORC1* (Vitamin K epoxide reductase complex subunit 1). It has been discovered that polymorphisms in the *VKORC1* gene Influence the individual's responsiveness to warfarin and other anticoagulant medications. Rs9923231, also known as -1639G>A or -1639G/C, is one of the *VKORC1* polymorphisms that has been the subject of the most research. This polymorphism, which is found in the *VKORC1* gene's promoter region, has been linked to changes in the dose requirements for warfarin. Rieder et al in 2005 conducted a study to analyze the effect of *VKORC1* haplotypes on the transcriptional control of the *VKORC1* gene and its effect on warfarin dose were examined in this work by Rieder et al. Specific *VKORC1* haplotypes, including those identified by the -1639G>A polymorphism, were discovered to be related to differences in the amount of warfarin that was needed, according to the study's findings. The G allele was connected to higher *VKORC1* expression and decreased sensitivity to warfarin, whereas the A allele was linked to lower *VKORC1* expression, increasing sensitivity to warfarin and requiring lower doses (Rieder et al., 2005). Geisen et al. Investigated the variation in oral anticoagulation caused by *VKORC1* haplotypes across individuals and between ethnic groups. The study examined the association between warfarin dose requirements in a broad patient group and *VKORC1* SNPs, including -1639G>A. The results showcased how *VKORC1* haplotypes exerted a significant

influence on the warfarin dosage among individuals and across different ethnic groups (Geisen et al., 2005).

1.4.1.3 *CYP2C9* AND *VKORC1* POLYMORPHISM IN OTHER POPULATION:

This study looked into the relationship between warfarin dosage requirements in a Swedish population and genetic differences in *CYP2C9* and *VKORC1*. The study's outcomes, underscoring the pivotal role of genetic factors in the optimization of warfarin dosing, unveiled a robust association between specific *CYP2C9* and *VKORC1* polymorphisms and the variability in warfarin dosage. (Wadelius et al., 2009). This study focuses on the prevalence of *CYP2C98* allele in African-Americans and its implications for pharmacogenetic dosing. The researchers found a high frequency of the *CYP2C98* allele in African-Americans, which is associated with reduced enzyme activity. This suggests that genetic testing for *CYP2C9* polymorphisms is important for personalized warfarin dosing in this population (Scott et al., 2013). Employing an international normalized ratio (INR)-centered dosage algorithm across diverse cohorts encompassing African, Caucasian, Chinese, and Indian populations, this study scrutinized the interethnic heterogeneity in warfarin maintenance dose prerequisites. The importance of population-specific genetic factors in warfarin dosing was highlighted by the researchers' discovery of considerable variations in *CYP2C9* and *VKORC1* allele frequencies among different populations (Lee et al., 2009). This study looked into the frequency of *CYP2C9* and *VKORC1* polymorphisms in Malaysia's various ethnic communities, including the Chinese, Malay, and Indian. The researchers discerned noteworthy alterations in genotypic compositions and allele frequencies among the populations, thereby signifying the presence of ethnic disparities in *CYP2C9* and *VKORC1* polymorphic profiles. This underscores the paramount importance of incorporating population-specific genetic variables into warfarin dosing algorithms. (Huang et al., 2015). The effect of *CYP2C9* and *VKORC1* polymorphisms on the likelihood of hemorrhagic complications in African-American and European-American warfarin-treated patients was investigated. The results underscored the importance of genetic testing for tailoring warfarin dosages to individual patients and elucidated how specific genetic polymorphisms in *CYP2C9* and *VKORC1* substantially heightened the risk of bleeding episodes in both ethnic cohorts (Limdi et al., 2008).

1.4.1.4 EARLIER STUDIES ABOUT *CYP2C9* AND *VKORC1* POLYMORPHISM AND WARFARIN:

Early research has looked on the relationship between *CYP2C9* polymorphisms and drug metabolism. The purpose of determining the allele frequency and genotype distribution of *CYP2C9* and *VKORC1* polymorphism in a population was to describe how the *CYP2C9* gene polymorphism affects structural and functional aspects of cells. In a baculovirus system, the researchers expressed both the wild-type and mutant variants of the *CYP2C9* enzyme and assessed their substrate selectivity. Comparing the I359L mutant version to the wild-type enzyme, they discovered that it had different substrate stereoselectivity. This study shed important light on how *CYP2C9* polymorphisms affect enzyme function (Zeldin et al., 1996). In this investigation, the relationship between *CYP2C9* polymorphisms and warfarin dosage requirements and bleeding complications risk was examined. The study team looked at a group of warfarin-treated patients and assessed their *CYP2C9* genotype. They discovered that those with particular *CYP2C9* polymorphisms needed lower dosages of warfarin and were more likely to experience bleeding problems. The significance of *CYP2C9* polymorphisms in predicting warfarin dose and adverse outcomes was highlighted by this study (Aithal et al., 1999). In this study, the impact of *CYP2C9* polymorphisms on the pharmacokinetics of the NSAID diclofenac was examined. When administering diclofenac to people with various *CYP2C9* genotypes, the researchers monitored the levels of the medication in blood. They discovered that neither the pharmacokinetics of diclofenac nor its inhibition of cyclooxygenases were affected by the *CYP2C9* genotype. According to this study, *CYP2C9* polymorphisms might not have a substantial impact on the metabolism of diclofenac (Kirchheiner et al., 2001).

In this particular study the relationship between *VKORC1* polymorphisms and warfarin dose requirements was examined in this study. A unique single nucleotide polymorphism (SNP) in the *VKORC1* gene (rs9923231), which was genotyped in a cohort of warfarin-treated patients, was found to be strongly linked with warfarin dose variability. When compared to patients with the wild-type allele, patients with the variant allele needed lower doses of warfarin. The significance of *VKORC1* polymorphisms in determining warfarin dose requirements was underlined by this study (Rieder et al., 2005). Erker, et al. reported that he links between *VKORC1* polymorphisms and warfarin sensitivity in a

Chinese population was examined in this study. They examined *VKORC1* SNPs in patients receiving warfarin treatment and contrasted their genotypes with those of the equilibrium-dosage and markedly fluctuating-dosage groups, respectively. It was ascertained that specific *VKORC1* haplotypes were associated with an elevated propensity for bleeding events and heightened sensitivity to warfarin. This investigation provided substantiating evidence for the impact of *VKORC1* polymorphisms on the response to warfarin within a specific ethnic demographic. (Erker et al., 2005). This study looked at the relationship between *VKORC1* polymorphisms and the amount of warfarin an Italian population needed to take. In patients on warfarin, the researchers genotyped *VKORC1* SNPs and evaluated how they related to consistent warfarin dosages. The investigation revealed a significant association between specific *VKORC1* mutations and the variability in warfarin dosage requirements. This study furnished supplementary evidence regarding the influence of *VKORC1* polymorphisms on warfarin dosage within a distinct demographic. (Ambrosio et al., 2005). The aim of this research was to establish the ideal warfarin starting dose for patients beginning anticoagulant medication. A randomized controlled experiment comparing two starting dosing approaches—fixed-dose initiation and a genotype-guided strategy employing *CYP2C9* and *VKORC1* genotyping—was carried out by the researchers. In comparison to fixed-dose beginning, they discovered that genotype-guided dosing resulted in less time to reach a stable therapeutic dose of warfarin (Steinberg et al., 2009). In another study by Vega, et al. conducted on individuals with nonvalvular atrial fibrillation, this study examined the efficiency and security of warfarin therapy in avoiding stroke. The results of a randomized controlled trial comparing aspirin and adjusted-dose warfarin revealed that warfarin was more useful at reducing the possibility of stroke and systemic embolism. This study proved warfarin's effectiveness in preventing strokes and supported its usage as the go-to treatment for atrial fibrillation (Vega et al., 1996).

CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS:

2.1.1 REAGENTS AND INSTRUMENT:

Warfarin Real-Time PCR Kit (Cat. No: 13r-10-03) (SNP Biotechnology R&D Ltd, Ankara, Turkey), DNA isolation kit (Hibrigen Biotechnology, Gebze/Kocaeli, Turkey), Real time PCR machine (HiMedia, Mumbai, India)

2.1.2 ACQUISITION OF SAMPLES:

The term "sample" describes a piece or specimen that is representative of a broader population or substance and is gathered and examined for scientific purposes. Participants' informed consent was obtained prior to collecting any samples. The study's goal, protocol, and any potential risks or advantages were all explicitly stated to the participants. The participants were given a chance to ask queries and explain any issues they may have. The ethical guidelines for human subject research published by Near East University were followed. The study procedure had been approved by the Near East University Ethics Committee. A sterile venipuncture technique was used to collect venous blood samples from 30 Turkish Cypriots from April to June. To prevent clotting and preserve the integrity of the DNA, proper blood collection tubes containing anticoagulants such as EDTA were utilized. To guarantee accurate monitoring and identification, each blood sample was labeled with patients name and age. Blood samples were treated with care in order to avoid contamination or degradation. The blood samples were stored at an appropriate temperature of 4^{o}C to -20^{o}C.

2.2 METHOD:

2.2.1 DNA ISOLATION:

Genomic DNA was isolated from blood samples using the Hibrigen DNA isolation kit. This involved cell lysis with a lysis buffer, followed by protein precipitation and removal. Genomic DNA was bound to a spin column's silica-based membrane using a binding buffer, and impurities were removed with wash buffers. The final pure genomic DNA was

assessed for concentration and purity using a spectrophotometer and stored at -20°C or -80°C.

2.2.2 MEASURING DNA CONCENTRATION:

Nanodrop spectrophotometer was used to measure the DNA concentration. The absorbance levels were measured at 260 and 280 nm wavelengths.

2.2.3 PREPARATION OF PCR REACTION MIXES:

Commercial Warfarin Real-Time PCR Kit (Cat. No: 13r-10-03) was used to detect *CYP2C9*2 (1639G>A), VKORC1 (C4302T)* mutations. Each isolated DNA was tested with wild type and mutant Real-Time Master mixes. The system provides reagents in a ready-to-use master-mix format which had been specifically adapted for 5' nuclease PCR using patented SNP analyses. The test system is designed for use with sequence specific primers and probe. The fluorescence of mutation analysis was FAM. Also, each master mix contains an internal control labeled with HEX/JOE dye. Different tubes were prepared for each mix. Before starting work, the master mixes were mixed gently by pipetting. In each sample, a volume of 20 µl of master mix was carefully dispensed using micropipettes equipped with sterile filter tips into individual optical white strips or tubes. Subsequently, 5 µl of DNA was introduced into each respective tube, and the subsequent experimental procedure was executed following the designated program.

2.2.4 PCR AMPLIFICATION AND GENOTYPING:

The Polymerase chain reaction mix was made in accordance with the instructions included with the warfarin real time PCR kit. The *CYP2C9* and *VKORC1* gene regions of interest were targeted with particular primer sets. PCR amplification was carried out using a real-time PCR instrument and the manufacturer's indicated cycling parameters. As the PCR progressed, the amplification of the *CYP2C9* and *VKORC1* gene areas was observed in real-time. To detect and measure the amplification of the necessary DNA sequences, special fluorescent probes contained in the Warfarin Real-Time PCR Kit were utilized. To create real-time PCR data, fluorescence signals were examined throughout each amplification cycle. The genotypes of the *CYP2C9* and *VKORC1* polymorphisms were determined using real-time PCR data. Each sample's genotyping was recorded, indicating the precise *CYP2C9* and *VKORC1* allele combinations present.

2.2.5 STATISTICAL ANALYSIS:

Chi-square test was used to analyze the allele frequencies and genotype distribution data Hardy-Weinberg equilibrium and other calculated parameters were utilized to examine departures from anticipated genotype frequencies. The obtained allele frequencies and genotype distribution were compared to data from different communities or databases. The findings were examined in terms of personalized medicine, drug response, and illness risk assessment in the Turkish Cypriot community.

CHAPTER III

RESULTS

3.1 RESULTS:

The table 3 given below provided in-depth insights into significant elements including age and gender distribution while offering a thorough summary of the overall characteristics of the study group. The table was designed with important statistical measures and significance values to make it easy to grasp the trends that were seen. Age was a significant factor in the study group, and the p-value of 0.039 presented demonstrated its statistical significance. The participants' average age was 40.1 years, with a 12.1-year standard deviation. This suggests that the participants' ages varied to some extent. The gender distribution within the study group was also highlighted by the table. It was clear that the study group was equally split between men and women, who make up 50% of all participants. Males had an average age of 45.8 years and a standard deviation of 14.2 years, while females had an average age of 36.3 years and a standard deviation of 9.2 years. The F-value of 4.705 was provided to evaluate the gender's possible influence on the study's variables. The variance between the means of various groups was indicated by the F-value. In this instance, it was hypothesized that there might be some variance in the traits under investigation between males and females.

Table 3: General Characteristic for the studied group

	N	**P-value**
Age	40.1±12.1	
Male	45.8±14.2	
Female	36.3±9.2	0.039

Gender	N (%)
Male	25 (50)
Female	25 (50)

F-value
4.705

3.1.1 *CYP2C9 G1639A:*

G1639A genetic variant is involved in the *CYP2C9* gene. The following are the potential changes. GG: The patient carries the G allele in two copies. GA: The patient carries one G allele copy and one A allele copy. AA: The patient carries the A allele in two copies. The genotype distribution of the *CYP2C9 G1639A* variant in Turkish Cypriot population is represented in a chart as GG: The observed count of people with the GG genotype is 20, whereas the predicted number is 17. GA: While there should be 24 people with the GA genotype, only 18 were found. AA: The observed count of people with the AA genotype is 12, when the predicted number is 9. Allele frequencies are represented as G: The G variation has an allele frequency of 0.580. This suggests that roughly 58% of the population under study carries the G allele. A: The A variant's allele frequency is 0.420. This shows that roughly 42% of the population under study carry the A allele. 3,408 is the Chi-squared value. A statistical technique called the Chi-squared test is used to examine if the observed and expected frequencies are significantly associated. A larger Chi-squared value in this situation denotes a bigger departure from the predicted genotype distribution. With regard to the Chi-squared test, the P-value is 0.648. The P-value is the likelihood that the genotype distribution reported could have been observed by pure chance. The observed genotype distribution is likely to be similar to the expected genotype distribution if the P-value is over the significance level, which is typically 0.05. Allele frequencies and observed and predicted genotype frequencies for the *CYP2C9 G1639A* variant in the population under study are detailed in the table. According to the Chi-squared test, there isn't a lot of variation from the predicted genotype distribution.

Table 5 Allele Frequency and genotype Distribution of CYP2C9 G1639 variant in studied populatio

Genotypes	**Expected**	**Observed**
GG	17	20
GA	24	18
AA	9	12
G	0.580	
A	0.420	
Chi-squared	3,408	

P-value	0.648

3.1.2 *VKORC1 C430T:*

This column below illustrates the several allele combinations (gene variants) that can occur at the particular location *"C430T"* in the *VKORC1* gene. According to various theoretical calculations or forecasts, this column indicated the estimated frequency of each genotype in the examined population. These calculations were frequently based on known genetic distributions or assumptions about the population being studied. This column displays the actual number of occurrences of each genotype found in the study population via genetic research. At position 430, this genotype had two copies of the "C" allele. Although 37 was the expected frequency, 38 was the observed frequency. At position 430, this genotype had one "C" and one "T" allele. The measured frequency was 10 and the predicted frequency was 12. At position 430, this genotype had two copies of the "T" allele. Although the expected frequency was one, the observed frequency was two. These two rows showed the frequency of the individual alleles ("C" and "T") at position 430 in the group investigated. The anticipated frequency of the "C" allele was 0.860, which means it will be found in around 86% of the population. The anticipated frequency of the "T" allele is 0.140, which means it will be found in around 14% of the population. The chi-squared value is a statistical measure used to detect whether there is a statistically significant discrepancy between predicted and observed frequencies. The estimated chi-squared value in this case was 1,435. A higher chi-squared value indicates a wider difference between expected and observed values. In this situation, the p-value was 0.230. Given the independence between the actual and predicted numbers, this p-value showed the likelihood of observing the genotype distribution (CC, CT, TT). Based on the Chi-squared test, the hypothesis that the observed and predicted distributions were significantly different was not strongly supported with a p-value of 0.230. This indicated that the genotype distribution of the *VKORC1 "C430T*" variant observed in the study population did not significantly deviate from the expected distribution.

Table 6 Allele Frequency and genotype Distribution of VKORC1 "C430T "variant in studied population

Genotypes	Expected	Observed
CC	37	38
CT	12	10
TT	1	2
C	0.860	
T	0.140	
Chi-squared	1,435	
P-value	0.230	

3.2 THE PERCENTAGE OF OBSERVED ALLELE FREQUENCY IN TURKISH CYPRIOT POPULATION:

The allele frequencies in Turkish Cypriot society for *CYP2C9 G1639A* variant was represented as the G variation had an allele frequency of 0.580. This suggested that roughly 58% of the Turkish Cypriot under study carries the G allele. The A variant had an allele frequency of 0.420. This shows that roughly 42% of the Turkish Cypriot under study carry the A allele. Moreover, the allele frequency in Turkish Cypriot individuals for *VKORC1 C430T* the "C" allele was projected to had a frequency of 0.860, signifying its presence in roughly 86% of the population. Conversely, the "T" allele was expected to had a frequency of 0.140, indicating its occurrence in approximately 14% of the population.

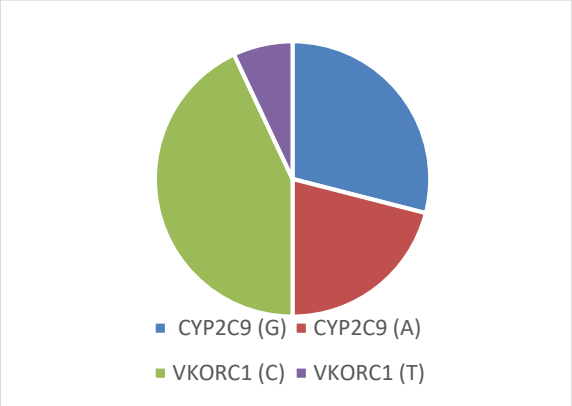
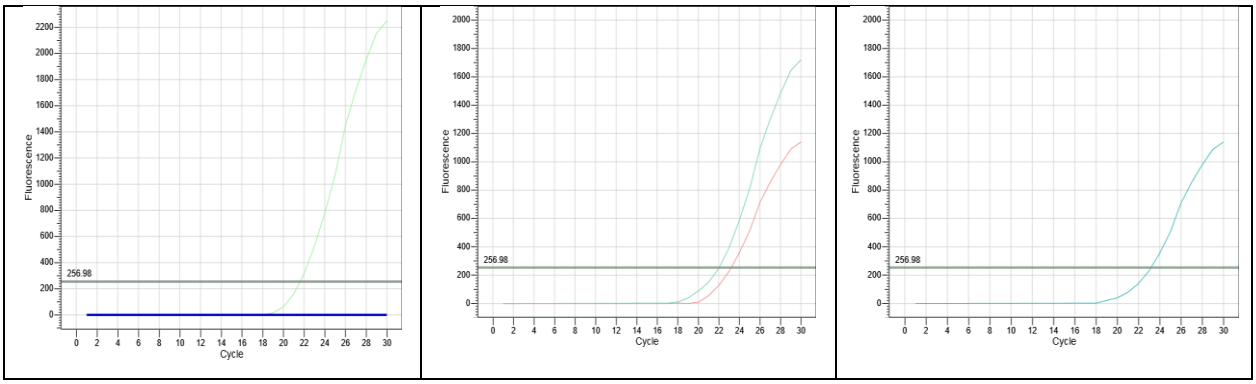


Figure 1: Showing percentage of Allele frequency

3.3 REAL TIME PCR CURVES OF DIFFERENT GENOTYPES:

3.3.1 *C430T*



2.1 *C430T CC* Homozygous	2.2 *C430T CT* Heterozygous	2.3 *C430T TT* Homozygous

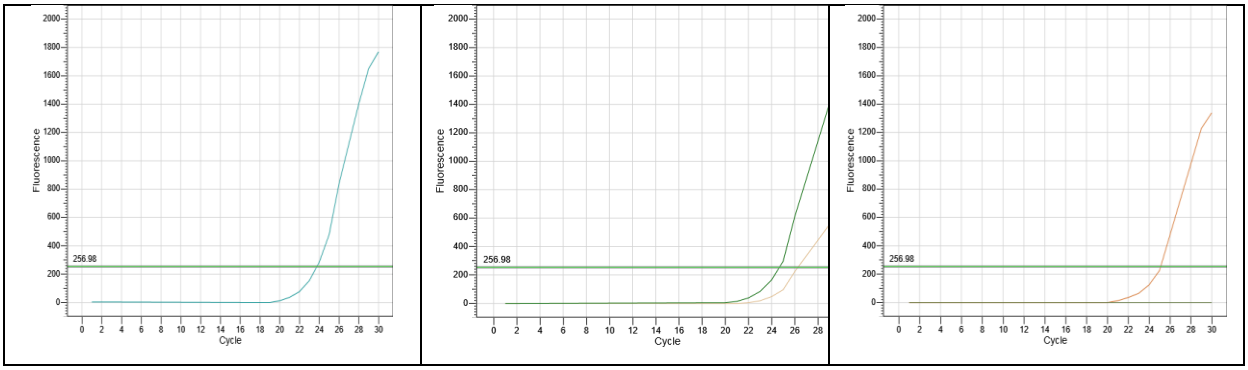
Figure 2: Real time PCR curve of *C430T*

In the above given figure 2.1 the curve represents that it was "*C430T CC* homozygous" which means the DNA sequence changes from a cytosine (C) base to a thymine (T) nucleotide at position 430. It was a wild type allele indicating that individuals had two copies of C allele at position 430 in *VKORC*1 gene.

In the above given figure 2.2 the green curve represents that it was "C" allele and the red curve represents that it was "T" allele which indicate that it was "*C430T CT* heterozygous" which means a unique genetic variation in which one of two copies (alleles) of a gene at a specific gene locus carries a cytosine base (C) at position 430 whereas the other allele carries a thymine base (T) at the same position. These individuals had intermediate sensitivity to warfarin.

The above give fig 2.3 the curve represents that it was "*C430T TT* homozygous" occurs when the thymine base (T) at position 430 occurs in both copies (alleles) of a gene at a given locus. It was mutant allele indicating that individuals had two copies of "T" allele at position 430 in *VKORC1* gene.

3.3.2 *G1629A*:



3.1 *G1639A AA* Homozygous	3.2 *G1639A GA* Heterozygous	3.3 *G1639A GG* Homozygous

Figure 3: Real time PCR curve of *G1639A:*

In the above given figure 3.1 the curve represents that it was "*G1639A AA* Homozygous" refers to a specific alteration in a gene's DNA sequence at location 1639. An Adenine (A) nucleotide takes the place of the original Guanine (G) nucleotide at this location. It was a mutant type indicating that individuals had two copies of "A" allele at position 1639.

In the above given figure 3.2 the green curve represents that it was "G" allele and red curve represents that it was "A" allele which indicate that it was "*G163A GA* Heterozygous refers to the precise mutation at position 1639 in the DNA sequence of a gene. At this location, an Adenine (A) nucleotide takes the place of the original Guanine (G) nucleotide. This indicates a person has one copy of the wild-type allele (G) and one copy of the mutant allele (A) at position 1639 in the *CYP2C9* gene.

In the above given figure 3.3 the curve represents that it was "*G1639A GG* Homozygous refers to the specific mutation in a gene's DNA sequence at position 1639. The G1639A mutation occurs when the initial Guanine (G) nucleotide at this location is changed to an Adenine (A) nucleotide. Both alleles in this instance carry the G1639A mutation, in which the original Guanine (G) nucleotide has been changed to an Adenine (A). This is a homozygous state, meaning that both alleles are the same. It was wild type indicating that individuals had two copies of "G" allele.

CHAPTER IV

DISCUSSION

4.1 *CYP2C9 G1639A* GENETIC VARIATION:

The *CYP2C9 G1639A* genetic variation involves a change from guanine (G) to adenine (A) nucleotide at position *1639* of the *CYP2C9* gene. The cytochrome P450 2C9 enzyme that metabolizes medications like warfarin and NSAIDs is encoded by this gene. Because the G1639A variant is linked to decreased enzyme activity, people with this variation may experience delayed drug metabolism and higher drug levels (Aithal et al., 1999). The prevalence of the *CYP2C9 G1639A* variant can vary among different populations for example in European populations, the *CYP2C9*2* variant was relatively common. It had been reported to be present in around 10-15% of individuals (Lee et al., 2002). The prevalence of the *CYP2C9*2* variant was lower in African populations with an estimated frequency of around 1-2% (Scott et al., 2007). The prevalence of *CYP2C9*2* can vary among Asian subpopulations. For example, in Chinese and Japanese populations, the frequency of this variant was reported to be around 4-7% (Matsushima et al., 2009). If we compare Turkish Cypriot population with European, Africans and Asian population indicated the expected frequency of each genotype in the population under study. The column then displayed the actual number of instances of each genotype found in the population under study using genetic analysis. GG (homozygous) The "G" allele was present in two copies at location 1639 in this genotype. While 17 was the predicted frequency, 20 was actually seen. GA (heterozygote) At location 1639, this genotype had both a "G" and an "A" allele. The observed frequency was 18, compared to the expected frequency of 24. AA (mutant homozygote) The "A" allele was present in two copies at location 1639 in this genotype. The observed frequency was 12, compared to the predicted frequency of 9. The table above provided information about the distribution of the *CYP2C9 "G1639"* genetic variant in the studied population, along with statistical values used to assess the significance of observed deviations from the expected distribution.

4.2 *VKORC1 C430T* GENETIC VARIATION:

The *C430T* genetic variant in the *VKORC1* gene signifies a single nucleotide polymorphism (SNP) characterized by the replacement of cytosine (C) with thymine (T)

at position 430. With regard to warfarin anticoagulant medication, this difference is particularly significant (Scott et al., 2013). The metabolism of the blood clot-preventing drug warfarin depends on the *VKORC1* gene. Because the *C430T* variant results in decreased enzyme activity, people with this variation may need to take lower doses of warfarin for successful anticoagulation (Arseneau et al., 2015). Personalized dosage regimens are informed by genetic testing. Consult with medical professionals with training in genetics or anticoagulation management for exact advice (Rieder et al., 2005). The table above showed the expected and actual frequencies of various genotypes in the population under study. The genotypes fall under the CC, CT, and TT categories. CC (homozygous) the expected frequency was 37, but the observed frequency was 38. CT (heterozygous) The expected frequency was 12, and the observed frequency was 10. TT (mutant homozygous) The expected frequency was 1, and the observed frequency was 2. Overall, it appears that the observed genotype distribution matches the predicted distribution.

4.3 GENOTYPE DISTRIBUTION:

The frequencies or ratios of various genotypes within a population or sample are referred to as genotype distribution. Polymorphisms in *CYP2C9* and *VKORC1* genes exhibit ethnic variability and can serve as essential factors for dosage adjustments in individuals undergoing warfarin treatment. In Turkish population who are quite similar to Turkish Cypriot population the genotype distribution of *CYP2C9*1* which was wild type was 61.72%. For *CYP2C9∗2* which was heterozygous mutant was 18.04%. And for *CYP2C9*3* heterozygous was 17.23%. The genotype distribution of *VKORC1* The frequency of the wild-type *VKORC1-1639 G/G* genotype was 28.8%, while the combined frequency of the heterozygous G/A and homozygous mutant A/A genotypes was 42.4% and 28.8%, respectively 9 (Aynacıoğlu et al., 2012). In the context of the *CYP2C9 G1639A* genetic variation within the Turkish Cypriot population, a comparative analysis between expected and observed genotype frequencies reveals noteworthy disparities. The GG genotype displayed an observed count of 20, surpassing the expected count of 17, indicating an elevated prevalence in the population. Conversely, the GA genotype exhibited an observed count of 18, falling below the anticipated value of 24, suggesting an underrepresentation relative to expectations. Furthermore, the AA genotype, with an

anticipated count of 9, was observed in 12 individuals, signifying an excess beyond the projected count. These findings underscore the genetic heterogeneity within the Turkish Cypriot population and emphasize the potential implications of these deviations for pharmacogenetic considerations and therapeutic responses, warranting further investigation to elucidate contributing factors. The observed frequency of individuals with the AA genotype exceeded the expected count, indicating a somewhat higher-than-anticipated prevalence of the AA genotype within the Turkish Cypriot population. In contrast, the GG genotype was generally overrepresented relative to the projected counts, while the GA genotype was underrepresented compared to expectations. These disparities in genotype distributions highlight the genetic heterogeneity present within the Turkish Cypriot population and emphasize the significance of these deviations in understanding pharmacogenetic variations and potential implications for therapeutic responses. Relative to the projected counts, the GG genotype was overrepresented, the GA genotype was underrepresented, and the AA genotype was somewhat overrepresented in the Turkish Cypriot population. In the case of the *VKORC1 C430T* genetic variation, the actual count for the CC genotype slightly exceeded the anticipated count of 37, indicating a marginally higher-than-expected prevalence of the CC genotype. Conversely, the observed count for the CT genotype was 10, in contrast to the projected value of 12, suggesting an underrepresentation of the CT genotype relative to expectations. The expected count for the TT genotype was 1, but the actual count was 2, signifying a slightly higher-than-expected prevalence of the TT genotype within the Turkish Cypriot population. These Within the Egyptian population, it had been determined that the prevalence of the *CYP2C9*2 allele stands at 12%, while the *CYP2C9*3 allele was estimated to be present in 6% of individuals. The *VKORC1 –1639AA* genotype and the frequency of the *VKORC1 –1639A* allele were calculated to be 22.9% and 42.7%, respectively (Manolopoulos., et al). This implies that roughly 23% of the examined population exhibits sensitivity to warfarin and would necessitate reduced warfarin dosages. Findings underscore the nuances in genotype distributions and their potential implications for pharmacogenetic considerations and therapeutic responses, warranting further exploration to discern the contributing factors. While the CT genotype was underrepresented in the observed count compared to the projected count, the observed counts for the CC and TT genotypes were

marginally greater than the expected counts. The genotype distribution of the *CYP2C9 G1639A* and *VKORC1 C430T* variations in the Turkish Cypriot population was revealed by these observations. A number of things, such as genetic drift, selection pressures, or restrictions on the sample size or study design, could cause variations from the projected counts. Larger sample numbers and additional research are required to confirm and comprehend these variations in genotype distribution. Comparing the results of Turkish Cypriot population with Turkish and Egyptians population it was clear that majority of Turkish and Turkish Cypriot population had near-to normal enzyme activity whereas in Egyptians some of the individuals require lower warfarin dosage.

4.4 ALLELE FREQUENCIES:

The relative distribution of various alleles within a population or sample is referred to as allele frequency. An allele is a gene's alternate form or version that resides in a particular region (locus) on a chromosome (Lewontin et al., 1974). The frequency of *CYP2C9*2 (G1639A)* was relatively common in some European and Caucasian populations, with frequencies ranging from approximately 10% to 15%. *CYP2C9*3 (A3603C)* The *3 allele was less common than *2 and was typically found at frequencies below 5% in European populations. *VKORC1*1* (wild type) is common, with frequencies close to 100% in some European populations. *VKORC1*2* (C1173T) allele was not that common with a frequency of almost 38% (Lee., et al). In a 2014 study involving a cohort of 148 healthy Greek-Cypriot individuals, the allele frequencies for *CYP2C9*2, *CYP2C9*3, and *-1639A* of *VKORC1* were determined to be 16.2%, 11.2%, and 53%, respectively (Nahar R., et al). In a separate 2012 study involving 557 English patients, individuals with the AA genotype at the -1639 position of the *VKORC1* gene, with a frequency of 14.5%, exhibited a lower warfarin dosage requirement to attain the desired International Normalized Ratio (INR) target. This genotype was associated with an increased risk of bleeding events, indicating heightened sensitivity to warfarin (Vargens., et al). Whereas in Turkish Cypriot population according to our findings which had an allele frequency of 0.580 for G and 0.420 for A, the G allele was more common than the A allele. This implies that for the *CYP2C9 G1639A* variation, the G allele was more prevalent in Turkish Cypriots. Similarly for Turkish Cypriot individuals for *VKORC1 C430T* variant an allele frequency of 0.140 for T and 0.860 for C, the C allele was more prevalent than the T allele. This suggested that

for the *VKORC1 C430T* variation, the C allele was more common in the population under study.

4.5 OUTCOME OF THIS STUDY:

This study describes the allele frequency and genotype distribution of the *CYP2C9 G1639A* variation in a population. The *CYP2C9* gene is involved in warfarin metabolism, and mutations in this gene can affect an individual's sensitivity to the medicine. To analyze the known correlations between *CYP2C9* genotypes and warfarin metabolism, The potential impact of allele frequency and genotype distribution on warfarin dosage requirements must be considered first. The *CYP2C9 G1639A* variation affects the CYP2C9 enzyme's enzymatic activity, which is involved in the metabolism of warfarin. Normal enzyme activity is connected with the GG genotype, reduced activity with the GA genotype, and the lowest activity with the AA genotype. The following observations can be drawn based on the genotypes observed in the examined population. The observed count of the GG genotype (20) surpasses the anticipated count (17), indicating an elevated prevalence of individuals exhibiting normal *CYP2C9* enzyme activity within the population. Conversely, the GA genotype demonstrates a lower observed count (18) compared to the expected count (24), signifying a reduced proportion of individuals with diminished *CYP2C9* enzyme activity. The observed count (12) for the AA genotype exceeds the predicted count (9). This implies a significantly higher prevalence of individuals with diminished *CYP2C9* enzyme activity. Based on these genotype distributions, it is conceivable that the examined population may comprise a relatively greater proportion of individuals exhibiting normal or near-normal *CYP2C9* enzyme activity Individuals with reduced *CYP2C9* enzyme activity (such as the GA and AA genotypes) often required lower warfarin doses to achieve the intended anticoagulant efficacy. Due to impaired warfarin metabolism, they exhibited heightened sensitivity to the medication.

This research also included data on the allele frequency and genotype distribution of the *VKORC1 C430T* variation in a population investigated. The *VKORC1* gene has been linked to an altered reaction to warfarin, an anticoagulant drug routinely used to prevent blood clots. In order to evaluate the potential impact of allele frequency and genotype

distribution on warfarin dose requirements, we must first consider the known connections between *VKORC1* genotypes and warfarin sensitivity. The *VKORC1 C430T* variation affects warfarin sensitivity through affecting the action of the VKORC1 enzyme, which is involved in the activation of vitamin K-dependent coagulation components that warfarin inhibits. The CC genotype is linked to normal enzyme function, the CT genotype to reduced function, and the TT genotype to the least function. The following observations can be made based on the genotypes observed in the examined population. The observed count for the CC genotype is slightly higher (38) than the expected count (37) for this genotype. These findings suggest a potential marginal increase in the population's proportion of individuals exhibiting normal *VKORC1* enzyme function, as the observed count for the CC genotype (38) slightly exceeds the anticipated count (37). Conversely, the CT genotype's recorded count (10) falls slightly below the expected count (12), indicating a slightly reduced prevalence of individuals with impaired *VKORC1* enzyme function. Moreover, the observed count for the TT genotype (2) surpasses the predicted count (1), implying a significantly higher prevalence of individuals characterized by the lowest *VKORC*1 enzyme function. Based on these genotype distributions, it was possible that the examined population contains a higher proportion of individuals with normal or near-normal *VKORC1* enzyme function. Individuals with diminished *VKORC1* enzyme function (such as the CT and TT genotypes) often require lower warfarin doses to obtain the appropriate anticoagulant effect. Because in Turkish Cypriot population, the average warfarin dosage required to achieved the desired anticoagulant effect might be higher compared to populations with a higher prevalence of reduced enzyme function genotypes (e.g., CT or TT genotypes). This was because individuals with normal enzyme function can metabolize and respond to warfarin more effectively, requiring slightly higher doses to achieve the same effect

4.6 COMPARISON OF ALELE FREQUENCY AND GENOTYPE DISTRIBUTION OF *CYP2C9* AND *VKORC1* GENE POLYMORPHISM IN TURKISH CYPRIOT POPULATION WITH OTHER POPULATIONS:

Varied populations have varied allele frequencies and genotype distributions for the *CYP2C9* and *VKORC1* genes. The estimated allele frequencies for *CYP2C92* and *CYP2C93* in European populations are 5–10% and 10-15%, respectively. The most

prevalent allele was the wild-type allele *(CYP2C91)*, which has an allele frequency of roughly 75-85%. The genotype *CYP2C91/1* is the most prevalent in Europeans, followed by *CYP2C91/2* and *CYP2C91/*3.* The allele frequencies of the *VKORC1 -1639G*>A polymorphism vary among populations in Europe. The G allele is more common, with frequencies ranging from 55-75%, while the A allele frequency is between 25 and 45%. The two genotypes that are most prevalent in Europeans are GG and GA constituting the majority (Wadelius, M. 2007). African populations have lower frequencies of the *CYP2C92* and *CYP2C93* alleles than European populations, with respective frequencies of 0-5% and less than 1%. African populations overwhelmingly exhibit *CYP2C9*1/*1* genotypes. African populations exhibit a lower frequency of the *VKORC1 -1639G*>A polymorphism compared to European populations. The frequency of the G allele is higher, ranging from 70 to 90%, while the frequency of the A allele is often lower, ranging from 10 to 30%. The most prevalent genotype is GG, followed by GA and AA genotypes (Scott et al., 2013). For *CYP2C92* and *CYP2C93*, Asian populations—including East Asians and South Asians—have different allele frequencies. The frequencies of the *CYP2C92* and *CYP2C93* alleles are often lower in East Asian populations, ranging from 0-5% and less than 1%, respectively. With *CYP2C92* allele frequencies ranging from 5–10% and *CYP2C93* allele frequencies hovering around 1-3%, South Asian groups have higher rates. The most prevalent genotype in populations from East and South Asia is *CYP2C9*1/*1*. Asian populations differ in the frequency of the *VKORC1 -1639G*>A allele. While the G allele frequency spans from 60 to 80%, East Asian populations have a higher prevalence of the A allele, which ranges from 20 to 40%. The G allele frequency is higher in South Asian populations, ranging from 70 to 90%, while the A allele frequency is lower, ranging from 10 to 30%. The most prevalent genotype among populations from East and South Asia is GG (Caldwell et al., 2008). When comparing the Turkish Cypriot population to European, African, and Asian populations, the observed percentages for the *CYP2C9 G1639A* variant revealed that the GG genotype was the most prevalent at 40%, followed by the GA genotype at 36%, and the AA genotype at 24%. Thus, in contrast to the mutant-type genotypes, the wild-type GG genotype exhibits a higher frequency in the Turkish Cypriot population. Similarly based on the observed percentages, the *VKORC1 C430T* variation was primarily represented by the CC genotype at 76%, followed by the CT

genotype at 20%, and the TT genotype at 4%. Consequently, the wild-type CC genotype prevails over the genotypes associated with mutant variations.

CHAPTER V

CONCLUSION

5. CONCLUSION:

In conclusion, the allele frequency and genotype distribution analysis of the *CYP2C9 G1639A* and *VKORC1 C430T* variation in this study provides insight into the genetic makeup of the Turkish Cypriot society in relation to warfarin metabolism. The observed distribution implies a greater prevalence of individuals exhibiting typical or closely approximate enzyme activity levels in both *CYP2C9* and *VKORC1*. These findings have implications for personalized medicine approaches and tailored warfarin dosing strategies, as individuals with diminished enzyme activity may require lower doses to attain the desired therapeutic effect. Understanding the genetic factors influencing warfarin metabolism can aid in optimizing medication regimens and minimizing the risk of adverse drug reactions in Turkish Cypriot individuals.

REFERENCES:

HosseinKhani, Z., Sadeghalvad, M., Norooznezhad, F., Khodarahmi, R., Fazilati, M., Mahnam, A., Fattahi, A., & Mansouri, K. (2018). The effect of CYP2C9*2, CYP2C9*3, and VKORC1-1639 G>A polymorphism in patients under warfarin therapy in city of Kermanshah. *Research in Pharmaceutical Sciences*, *13*(4), 377. https://doi.org/10.4103/1735-5362.235165

Bagher, A. M. (2023). Association of CYP2C9∗3 and CYP2C8∗3 Non-Functional Alleles with Ibuprofen-Induced Upper Gastrointestinal Toxicity in a Saudi Patient. *Case Reports in Medicine*, *2023*, 1–6. https://doi.org/10.1155/2023/6623269

Zanger, U. M., & Schwab, M. (2013b). Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & Therapeutics*, *138*(1), 103–141. https://doi.org/10.1016/j.pharmthera.2012.12.007

Sandberg, M., Yasar, U., Strömberg, P., Höög, J., & Eliasson, E. (2002). Oxidation of celecoxib by polymorphic cytochrome P450 2C9 and alcohol dehydrogenase. *British Journal of Clinical Pharmacology*, *54*(4), 423–429. https://doi.org/10.1046/j.1365-2125.2002.01660.x

Aithal, G. P., Day, C. P., Kesteven, P., & Daly, A. K. (1999). Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *The Lancet*, *353*(9154), 717–719. https://doi.org/10.1016/s0140-6736(98)04474-2

Hodgson, D. A., Feldberg, I., Sharp, N., Cronin, N. J., Evans, M., & Hirschowitz, L. (1999). Microwave endometrial ablation: development, clinical trials and outcomes at three years. *Bjog: An International Journal of Obstetrics and Gynaecology*, *106*(7), 684–694. https://doi.org/10.1111/j.1471-0528.1999.tb08368.x

Neyman, J., & Pearson, E. S. (1928). ON THE USE AND INTERPRETATION OF CERTAIN TEST CRITERIA FOR PURPOSES OF STATISTICAL INFERENCE. *Biometrika*, *20A*(3–4), 263–294. https://doi.org/10.1093/biomet/20a.3-4.263

Fisher, R. A. (1945). Statistical methods for research workers. *American Journal of Clinical Pathology*, *15*(11), 552.3-552. https://doi.org/10.1093/ajcp/15.11.552b

Nadaraya, E. A. (1964). On estimating regression. *Theory of Probability and Its Applications*, *9*(1), 141–142. https://doi.org/10.1137/1109020

Nei, M. (1987). Molecular Evolutionary Genetics. In *Columbia University Press eBooks*. https://doi.org/10.7312/nei-92038

Wray, G. A., Hahn, M. W., Abouheif, E., Balhoff, J. P., Pizer, M., Rockman, M. V., & Romano, L. (2003). The evolution of transcriptional regulation in Eukaryotes. *Molecular Biology and Evolution*, *20*(9), 1377–1419. https://doi.org/10.1093/molbev/msg140

Hardy, G. H. (1908). Mendelian proportions in a mixed population. *Molecular Genetics and Genomics*. https://doi.org/10.1007/bf01990610

Bolaji, U. O., Ajasa, A. A., Ahmed, R. O., Bello, S. S., & Ositanwosu, O. E. (2021). Cattle Conservation in the 21st Century: A mini review. *Open Journal of Animal Sciences*, *11*(02), 304–332. https://doi.org/10.4236/ojas.2021.112023

Hedrick, P. W. (1983). *Genetics of populations*. http://lib.tums.ac.ir/site/catalogue/49135

Hamberg, A., Ml, D., Barban, M., Scordo, M. G., Wadelius, M., Pengo, V., Padrini, R., & Jonsson, E. N. (2007). A PK–PD model for predicting the impact of age, CYP2C9, and VKORC1 genotype on individualization of Warfarin therapy. *Clinical Pharmacology & Therapeutics*, *81*(4), 529–538. https://doi.org/10.1038/sj.clpt.6100084

Fihn, S. D., Callahan, C. L., Martin, D. C., McDonell, M. B., Henikoff, S., & White, R. (1996). The Risk for and Severity of Bleeding Complications in Elderly Patients Treated with Warfarin. *Annals of Internal Medicine*, *124*(11), 970. https://doi.org/10.7326/0003-4819-124-11-199606010-00004

Kirchheiner, J., Meineke, I., Steinbach, N., Meisel, C., Roots, I., & Brockmöller, J. (2003). Pharmacokinetics of diclofenac and inhibition of cyclooxygenases 1 and 2: no relationship to the CYP2C9 genetic polymorphism in humans. *British Journal of Clinical Pharmacology*, *55*(1), 51–61. https://doi.org/10.1046/j.1365-2125.2003.01712.x

.

Crowther, M., Ageno, W., & Garcia, D. (2009). Oral Vitamin K Versus Placebo to Correct Excessive Anticoagulation in Patients Receiving Warfarin: A Randomized Trial. *Journal of Vascular Surgery*, *50*(1), 233. https://doi.org/10.1016/j.jvs.2009.05.042

D'Andrea, G., D'Ambrosio, R. L., P, P., Chetta, M., Santacroce, R., Brancaccio, V., Grandone, E., & Margaglione, M. (2005). A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. *Blood*, *105*(2), 645–649. https://doi.org/10.1182/blood-2004-06-2111

Yuan, H., Chen, J., Lee, M. M., Wung, J., Chen, Y., Charng, M., Lu, M., Hung, C., Wei, C., Chen, C., Wu, J., & Chen, Y. (2005). A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. *Human Molecular Genetics*, *14*(13), 1745–1751. https://doi.org/10.1093/hmg/ddi180

Rieder, M. J., Reiner, A. P., Gage, B. F., Nickerson, D. A., Eby, C. S., McLeod, H. L., Blough, D. K., Thummel, K. E., Veenstra, D. L., & Rettie, A. E. (2005d). Effect of*VKORC1*Haplotypes on Transcriptional Regulation and Warfarin Dose. *The New England Journal of Medicine*, *352*(22), 2285–2293. https://doi.org/10.1056/nejmoa044503

Haining, R. L., Hunter, A. P., Veronese, M. E., Trager, W., & Rettie, A. E. (1996). Allelic variants of human cytochrome P450 2C9: Baculovirus-Mediated Expression, Purification, Structural Characterization, substrate stereoselectivity, and Prochiral selectivity of the Wild-Type and I359L mutant forms. *Archives of Biochemistry and Biophysics*, *333*(2), 447–458. https://doi.org/10.1006/abbi.1996.0414

Limdi, N., McGwin, G., Goldstein, J., Beasley, T. M., Arnett, D., Adler, B., Baird, M., & Rt, A. (2007). Influence of CYP2C9 and VKORC1 1173C/T genotype on the risk of hemorrhagic complications in African-American and European-American patients on Warfarin. *Clinical Pharmacology & Therapeutics*, *83*(2), 312–321. https://doi.org/10.1038/sj.clpt.6100290

Niu, X., Liu, S., Li, J., & Chen, J. (2015). Role of MIR-3619-5P in B-Catenin-Mediated Non-Small Cell Lung Cancer Growth and Invasion. *Cellular Physiology and Biochemistry*, *37*(4), 1527–1536. https://doi.org/10.1159/000438520

Gomes, T., Mamdani, M., & Juurlink, D. N. (2009). Macrolide-Induced Digoxin Toxicity: A Population-Based Study. *Clinical Pharmacology & Therapeutics*, *86*(4), 383–386. https://doi.org/10.1038/clpt.2009.127

Scott, S. A., Jaremko, M., Lubitz, S. A., Kornreich, R., Halperin, J. L., & Desnick, R. J. (2009). *CYP2C9*8* is prevalent among African–Americans: implications for pharmacogenetic dosing. *Pharmacogenomics*, *10*(8), 1243–1255. https://doi.org/10.2217/pgs.09.71

Caldwell, M. D., Awad, T., Johnson, J. A., Gage, B. F., Falkowski, M., Gardina, P. J., Hubbard, J. C., Turpaz, Y., Langaee, T., Eby, C. S., King, C. R., Brower, A., Schmelzer, J. R., Glurich, I., Vidaillet, H., Yale, S. H., Zhang, K. Q., Berg, R. L., & Burmester, J. K. (2008). CYP4F2 genetic variant alters required warfarin dose. *Blood*, *111*(8), 4106–4112. https://doi.org/10.1182/blood-2007-11-122010

.

Bounameaux, H., & Reber, G. (2010). New oral antithrombotics: a need for laboratory monitoring. Against. *Journal of Thrombosis and Haemostasis*, *8*(4), 627–630. https://doi.org/10.1111/j.1538-7836.2010.03759.x

Hussein, M. N. A., Böing, A. N., Sturk, A., Hau, C. M., & Nieuwland, R. (2007). Inhibition of microparticle release triggers endothelial cell apoptosis and detachment. *Thrombosis and Haemostasis*, *98*(11), 1096–1107. https://doi.org/10.1160/th05-04-0231

Lee, S., Ng, S. B., Oldenburg, J., Chong, P. K., Rost, S., Guo, J., Yap, H., Rankin, S., Khor, H., & Yeo, T. (2006). Interethnic variability of warfarin maintenance requirement is explained by VKORC1 genotype in an Asian population. *Clinical Pharmacology & Therapeutics*, *79*(3), 197–205. https://doi.org/10.1016/j.clpt.2005.11.006

Aquilante, C. L., Langaee, T., Lopez, L. M., Yarandi, H., Tromberg, J., Mohuczy, D., Gaston, K., Waddell, C. D., Chirico, M. J., & Johnson, J. A. (2006). Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and

cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. *Clinical Pharmacology & Therapeutics*, *79*(4), 291–302. https://doi.org/10.1016/j.clpt.2005.11.011

Higashi, M. K. (2002). Association between CYP2C9 genetic variants and Anticoagulation-Related outcomes during Warfarin therapy. *JAMA*, *287*(13), 1690. https://doi.org/10.1001/jama.287.13.1690

Johnson, J. A., Caudle, K., Gong, L., Whirl-Carrillo, M., Stein, C. M., Scott, S. A., Lee, M., Gage, B. F., Kimmel, S., Perera, M. A., Anderson, J. S., Pirmohamed, M., Klein, T., Limdi, N., Cavallari, L., & Wadelius, M. (2017). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 update. *Clinical Pharmacology & Therapeutics*, *102*(3), 397–404. https://doi.org/10.1002/cpt.668

Caudle, K. E., Dunnenberger, H. M., Freimuth, R. R., Peterson, J. F., Burlison, J. D., Whirl-Carrillo, M., Scott, S. A., Rehm, H. L., Williams, M. S., Klein, T. E., Relling, M. V., & Hoffman, J. M. (2017). Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC). *Genetics in Medicine*, *19*(2), 215–223. https://doi.org/10.1038/gim.2016.87

ASCPT News. (2013). *Clinical Pharmacology & Therapeutics*, *93*(6), 462–465. https://doi.org/10.1038/clpt.2013.64

André, F., Ciccolini, J., Spano, J., Penault-Llorca, F., Mounier, N., Freyer, G., Blay, J., & Milano, G. (2013). Personalized medicine in oncology: where have we come from and where are we going? *Pharmacogenomics*, *14*(8), 931–939. https://doi.org/10.2217/pgs.13.79

Zanger, U. M., & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & Therapeutics*, *138*(1), 103–141. https://doi.org/10.1016/j.pharmthera.2012.12.007

Relling, M. V., & Evans, W. E. (2015). Pharmacogenomics in the clinic. *Nature*, *526*(7573), 343–350. https://doi.org/10.1038/nature15817

Kealey, C., Chen, Z., Christie, J. D., Thorn, C. F., Whitehead, A. S., Price, M., Samaha, F. F., & Kimmel, S. E. (2007). Warfarin and cytochrome P450 2C9 genotype: possible ethnic variation in warfarin sensitivity. *Pharmacogenomics*, *8*(3), 217–225. https://doi.org/10.2217/14622416.8.3.217

Ansell, J., Hirsh, J., Hylek, E. M., Jacobson, A., Crowther, M., & Palareti, G. (2008). Pharmacology and management of the vitamin K antagonists. *Chest*, *133*(6), 160S-198S. https://doi.org/10.1378/chest.08-0670

Ageno, W., Gallus, A., Wittkowsky, A. K., Crowther, M., Hylek, E. M., & Palareti, G. (2012). Oral anticoagulant therapy. *Chest*, *141*(2), e44S-e88S. https://doi.org/10.1378/chest.11-2292

Mv, R., & Klein, T. (2011). CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clinical Pharmacology & Therapeutics*, *89*(3), 464–467. https://doi.org/10.1038/clpt.2010.279

Arseneau, K. O., & Cominelli, F. (2014). Targeting leukocyte trafficking for the treatment of inflammatory bowel disease. *Clinical Pharmacology & Therapeutics*, *97*(1), 22–28. https://doi.org/10.1002/cpt.6

Bullinger, L., Döhner, K., Kranz, R., Stirner, C., Fröhling, S., Scholl, C., Kim, Y. H., Schlenk, R. F., Tibshirani, R., Döhner, H., & Pollack, J. R. (2008). An FLT3 gene-expression signature predicts clinical outcome in normal karyotype AML. *Blood*, *111*(9), 4490–4495. https://doi.org/10.1182/blood-2007-09-115055

Hicks, J. K., Sangkuhl, K., Swen, J. J., Ellingrod, V. L., Müller, D., Saito, K., Bishop, J. R., Kharasch, E. D., Skaar, T. C., Gaedigk, A., Dunnenberger, H. M., Klein, T. E., Caudle, K. E., & Stingl, J. C. (2017). Clinical pharmacogenetics implementation consortium guideline (CPIC) for *CYP2D6* and *CYP2C19* genotypes and dosing of tricyclic antidepressants: 2016 update. *Clinical Pharmacology & Therapeutics*, *102*(1), 37–44. https://doi.org/10.1002/cpt.597

Pratt, V. M., Cavallari, L. H., Del Tredici, A. L., Hachad, H., Ji, Y., Kalman, L. V., Ly, R. C., Moyer, A. M., Scott, S. A., Whirl-Carrillo, M., & Weck, K. E. (2020). Recommendations for clinical warfarin genotyping allele selection. *The Journal*

of Molecular Diagnostics, *22*(7), 847–859. https://doi.org/10.1016/j.jmoldx.2020.04.204

Rieder, M. J., Reiner, A. P., Gage, B. F., Nickerson, D. A., Eby, C. S., McLeod, H. L., Blough, D. K., Thummel, K. E., Veenstra, D. L., & Rettie, A. E. (2005). Effect of*VKORC1*Haplotypes on Transcriptional Regulation and Warfarin Dose. *The New England Journal of Medicine*, *352*(22), 2285–2293. https://doi.org/10.1056/nejmoa044503

Takeuchi, F., McGinnis, R., Bourgeois, S., Barnes, C., Eriksson, N., Soranzo, N., Whittaker, P., Ranganath, V., Kumanduri, V., McLaren, W., Holm, L. W., Lindh, J. D., Rane, A., Wadelius, M., & Deloukas, P. (2009). A Genome-Wide Association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of Warfarin dose. *PLOS Genetics*, *5*(3), e1000433. https://doi.org/10.1371/journal.pgen.1000433

Oral Session III-B (OIII-B) Molecular Targets and Genetics in Oncology 9:30 am – 10:30 am. (2011). *Clinical Pharmacology & Therapeutics*, *89*, S66–S68. https://doi.org/10.1038/clpt.2010.334

Finishing the euchromatic sequence of the human genome. (2004). *Nature*, *431*(7011), 931–945. https://doi.org/10.1038/nature03001

Vasiljević, P., Živković, J., Vukelić-Nikolić, M. Đ., & Najman, S. (2017). Determining the Biological Properties of Biomaterials In Vivo. In *Springer eBooks* (pp. 477–499). https://doi.org/10.1007/978-3-319-68025-5_17

.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A., Dvorkin, M., Kulikov, A. S., Lesin, V., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPADES: A new genome assembly algorithm and its applications to Single-Cell sequencing. *Journal of Computational Biology*, *19*(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

Mercer, T. R., Dinger, M. E., & Mattick, J. S. (2009). Long non-coding RNAs: insights into functions. *Nature Reviews Genetics*, *10*(3), 155–159. https://doi.org/10.1038/nrg2521

Shlyueva, D., Stampfel, G., & Stark, A. (2014). Transcriptional enhancers: from properties to genome-wide predictions. *Nature Reviews Genetics*, *15*(4), 272–286. https://doi.org/10.1038/nrg3682

Novembre, J., & Barton, N. (2018). Tread lightly interpreting polygenic tests of selection. *Genetics*, *208*(4), 1351–1355. https://doi.org/10.1534/genetics.118.300786

Hancock, A. M., Witonsky, D. B., Gordon, A. S., Eshel, G., Pritchard, J. K., Coop, G., & Di Rienzo, A. (2008). Adaptations to climate in candidate genes for common metabolic disorders. *PLOS Genetics*, *4*(2), e32. https://doi.org/10.1371/journal.pgen.0040032

Rosenberg, N. A., Pritchard, J. K., Weber, J. L., Cann, H. M., Kidd, K. K., Zhivotovsky, L. A., & Feldman, M. W. (2002). Genetic structure of human populations. *Science*, *298*(5602), 2381–2385. https://doi.org/10.1126/science.1078311

Sarig, O., & Sprecher, E. (2017). The Molecular Revolution in Cutaneous Biology: Era of Next-Generation Sequencing. *Journal of Investigative Dermatology*, *137*(5), e79–e82. https://doi.org/10.1016/j.jid.2016.02.818

Sherry, S. T., Ward, M., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., & Sirotkin, K. (2001). dbSNP: the NCBI database of genetic variation. *Nucleic Acids Research*, *29*(1), 308–311. https://doi.org/10.1093/nar/29.1.308

MacDonald, Ziman, R., Yuen, R. K. C., Feuk, L., & Scherer, S. W. (2013). The Database of Genomic Variants: a curated collection of structural variation in the human genome. *Nucleic Acids Research*, *42*(D1), D986–D992. https://doi.org/10.1093/nar/gkt958

Buschiazzo, E., & Gemmell, N. J. (2006). The rise, fall and renaissance of microsatellites in eukaryotic genomes. *BioEssays*, *28*(10), 1040–1050. https://doi.org/10.1002/bies.20470

Tishkoff, S. A., & Kidd, K. K. (2004). Implications of biogeography of human populations for “race” and medicine. *Nature Genetics*, *36*(S11), S21–S27. https://doi.org/10.1038/ng1438

Henn, B. M., Cavalli-Sforza, L., & Feldman, M. W. (2012). The great human expansion. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(44), 17758–17764. https://doi.org/10.1073/pnas.1212380109

Hindorff, L. A., Bonham, V. L., Brody, L. C., Ginoza, M., Hutter, C. M., Manolio, T. A., & Green, E. D. (2017). Prioritizing diversity in human genomics research. *Nature Reviews Genetics*, *19*(3), 175–185. https://doi.org/10.1038/nrg.2017.89

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., McCarthy, M. I., Ramos, E. M., Cardon, L. R., Chakravarti, A., Cho, J. H., Guttmacher, A. E., Kong, A., Kruglyak, L., Mardis, E. R., Rotimi, C. N., Slatkin, M., Valle, D., Whittemore, A. S., . . . Visscher, P. M. (2009). Finding the missing heritability of complex diseases. *Nature*, *461*(7265), 747–753. https://doi.org/10.1038/nature08494

Hizel, H. C. (2017). Highly personalized reports for personalized drug selection by expert systems as clinical decision support. *Personalized Medicine*, *14*(2), 93–97. https://doi.org/10.2217/pme-2016-0083

W, J., Geng, S., Wang, S. B., Zhang, G. L., Fu, W., & Shu, B. (2011). Genetic diversity of the newly invasive weed Flaveria bidentis (Asteraceae) reveals consequences of its rapid range expansion in northern China. *Weed Research*, *51*(4), 363–372. https://doi.org/10.1111/j.1365-3180.2011.00856.x

Marks, J. (1988). Molecular evolutionary genetics. By M. Nei. New York: Columbia University Press. 1987. x + 512 pp., tables, figures, indexes. $50.00 (cloth). *American Journal of Physical Anthropology*, *75*(3), 428–429. https://doi.org/10.1002/ajpa.1330750317

Emahazion, T., Jobs, M., Howell, W. M., Siegfried, M., Wyöni, P., Prince, J. A., & Brookes, A. J. (1999). Identification of 167 polymorphisms in 88 genes from candidate neurodegeneration pathways. *Gene*, *238*(2), 315–324. https://doi.org/10.1016/s0378-1119(99)00330-3

Redon, R., Ishikawa, S., Fitch, K. R., Feuk, L., Perry, G. H., Andrews, T. D., Fiegler, H., Shapero, M. H., Carson, A. R., Chen, W., Cho, E. K., Dallaire, S., Freeman, J. L., González, J. R., Gratacòs, M., Huang, J., Kalaitzopoulos, D. R., Komura, D., MacDonald, . . . Hurles, M. E. (2006). Global variation in copy number in the

human genome. *Nature*, *444*(7118), 444–454. https://doi.org/10.1038/nature05329

Stefansson, H., Rujescu, D., Cichon, S., Pietiläinen, O., Ingason, A., Steinberg, S., Fossdal, R., Sigurdsson, E., Sigmundsson, T., Buizer-Voskamp, J. E., Hansen, T., Jakobsen, K. D., Muglia, P., Francks, C., Matthews, P. M., Gylfason, A., Halldorsson, B. V., Gudbjartsson, D. F., Thorgeirsson, T. E., . . . Stefansson, K. (2008). Large recurrent microdeletions associated with schizophrenia. *Nature*, *455*(7210), 232–236. https://doi.org/10.1038/nature07229

Büller, H. R., Agnelli, G., Hull, R., Hyers, T. M., Prins, M. H., & Raskob, G. E. (2004). Antithrombotic therapy for venous thromboembolic disease. *Chest*, *126*(3), 401S-428S. https://doi.org/10.1378/chest.126.3_suppl.401s

.

Dager, W. E., Branch, J. M., King, J. H., White, R., Quan, R. S., Musallam, N. A., & Albertson, T. E. (2000). Optimization of inpatient warfarin therapy: Impact of daily consultation by a Pharmacist-Managed Anticoagulation Service. *Annals of Pharmacotherapy*, *34*(5), 567–572. https://doi.org/10.1345/aph.18192

Lander, E. S., Linton, L., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M. J., Fitzhugh, W. W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J. G., Kann, L., Lehoczky, J. A., Levine, R., McEwan, P., . . . Frazier, M. (2001). Initial sequencing and analysis of the human genome. *Nature*, *409*(6822), 860–921. https://doi.org/10.1038/35057062

Wu, C., & Arora, P. (2016). Noncoding Genome-Wide Association Studies Variant for obesity: Inroads into mechanism. *Journal of the American Heart Association*, *5*(7). https://doi.org/10.1161/jaha.115.003060

Relling, M. V., & Evans, W. E. (2015). Pharmacogenomics in the clinic. Nature, 526(7573), 343-350.

Dieri, R. A., De Laat, B., & Hemker, H. (2012). Thrombin generation: What have we learned? *Blood Reviews*, *26*(5), 197–203. https://doi.org/10.1016/j.blre.2012.06.001

Vanakker, O., Martin, L., Gheduzzi, D., Leroy, B. P., Loeys, B., Guerci, V. I., Matthys, D., Terry, S. F., Coucke, P., Pasquali-Ronchetti, I., & De Paepe, A. (2007).

Pseudoxanthoma Elasticum-Like Phenotype with Cutis Laxa and Multiple Coagulation Factor Deficiency Represents a Separate Genetic Entity. *Journal of Investigative Dermatology*, *127*(3), 581–587. https://doi.org/10.1038/sj.jid.5700610

Swen, J. J., Nijenhuis, M., De Boer, A., Grandia, L., Zee, A. M. D., Mulder, H., Rongen, G. A., Van Schaik, R. H., Schalekamp, T., Touw, D., Van Der Weide, J., Wilffert, B., Deneer, V. H., & Guchelaar, H. (2011). Pharmacogenetics: From Bench to Byte— An Update of Guidelines. *Clinical Pharmacology & Therapeutics*, *89*(5), 662–673. https://doi.org/10.1038/clpt.2011.34

Mv, R., & Klein, T. (2011b). CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clinical Pharmacology & Therapeutics*, *89*(3), 464–467. https://doi.org/10.1038/clpt.2010.279

Kisor, D. F., & Bodzin, A. S. (2018). Pharmacogenomics in liver transplantation: testing the recipient and the *ex-vivo* donor liver. *Pharmacogenomics*, *19*(9), 753–756. https://doi.org/10.2217/pgs-2018-0052

Kon, S. S., Canavan, J. L., & Man, W. D. (2012). Pulmonary rehabilitation and acute exacerbations of COPD. *Expert Review of Respiratory Medicine*. https://doi.org/10.1586/ers.12.47

Pirmohamed, M., Burnside, G., Eriksson, N., Jorgensen, A., Toh, C. H., Nicholson, T., Kesteven, P., Christersson, C., Wahlström, B., Stafberg, C., Zhang, J. E., Leathart, J., Kohnke, H., Zee, A. M. D., Williamson, P., Daly, A. K., Avery, P., Kamali, F., Wadelius, M., & Act, A. (2013). A randomized trial of Genotype-Guided dosing of warfarin. *The New England Journal of Medicine*, *369*(24), 2294–2303. https://doi.org/10.1056/nejmoa1311386

Rieder, M. J., Reiner, A. P., Gage, B. F., Nickerson, D. A., Eby, C. S., McLeod, H. L., Blough, D. K., Thummel, K. E., Veenstra, D. L., & Rettie, A. E. (2005b). Effect of*VKORC1*Haplotypes on Transcriptional Regulation and Warfarin Dose. *The New England Journal of Medicine*, *352*(22), 2285–2293. https://doi.org/10.1056/nejmoa044503

Yiu, Z. Z. N., & Warren, R. B. (2017). Raising standards for the evaluation of future psoriasis therapeutics: A critical checklist. *Clinical Pharmacology & Therapeutics*, *102*(4), 642–648. https://doi.org/10.1002/cpt.788

Huang, S. M., Abernethy, D. R., Wang, Y., Zhao, P., & Zineh, I. (2013). The utility of modeling and simulation in drug development and regulatory review. *Journal of Pharmaceutical Sciences*, *102*(9), 2912–2923. https://doi.org/10.1002/jps.23570

Rieder, M. J., Reiner, A. P., Gage, B. F., Nickerson, D. A., Eby, C. S., McLeod, H. L., Blough, D. K., Thummel, K. E., Veenstra, D. L., & Rettie, A. E. (2005c). Effect of*VKORC1*Haplotypes on Transcriptional Regulation and Warfarin Dose. *The New England Journal of Medicine*, *352*(22), 2285–2293. https://doi.org/10.1056/nejmoa044503

Hindorff, L. A., Heckbert, S. R., Smith, N. L., Marciante, K. D., & Psaty, B. M. (2007). Common VKORC1 variants are not associated with arterial or venous thrombosis. *Journal of Thrombosis and Haemostasis*, *5*(10), 2025–2027. https://doi.org/10.1111/j.1538-7836.2007.02705.x