AMINE BEKAI

INVESTIGATING hERG GENE EXPRESSION LEVELS IN CORONARY ARTERY DISEASE PATIENTS

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M.Sc. THESIS

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We certify that we have read the thesis submitted by Ms Amine Bekai titled "**Investigating hERG Gene Expression Levels in Coronary Artery Disease**" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Medical Biology and Genetics.

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I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Amine Bekai
25/06/2024

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Amine Bekai

Abstract

Investigating hERG Expression Levels in Coronary Artery Disease

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Abstract:

The α-subunits of the human ether-à-go-go-related gene (hERG) potassium channel, which is essential for cardiac repolarization, are encoded by the hERG1a and hERG1b genes. Variations in the way these genes are expressed have been connected to a number of heart conditions, such as coronary artery disease (CAD). Using quantitative real-time PCR (qPCR), this study sought to determine the RNA expression levels of hERG1a and hERG1b in a cohort of 32 CAD patients and 36 healthy controls.

Peripheral blood samples were used to extract total RNA, which was then reverse transcribed. The resultant cDNA was then used for qPCR analysis using primers specific to hERG1a, hERG1b, and a reference gene. Utilizing the 2^-ΔΔCt technique, the target genes were relative quantified. To compare the RNA levels between the patient and control groups, statistical analysis was done.

The findings revealed that CAD patients' expression of hERG1a and hERG1b were significantly lower than that of healthy controls ($p<0.05$). These results imply that the pathophysiology of coronary artery disease may be influenced by the imbalance in the expression of the hERG1a and hERG1b isoforms. Also, Patients showed significantly longer QT and QTc intervals compared to the control group. To clarify the underlying mechanisms and possible therapeutic implications of these changes in gene expression in the context of CAD, more research is necessary.

***Key Words**: **Co**ronary artery disease, Long QT-Syndrome ion channels, hERG1a, hERG1b, gene expression levels.*

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List of Abbreviations

ACEI:	Angiotensin-Converting Enzyme Inhibitors
ACS:	Acute Coronary Syndrome
AHR:	Transcriptional Factor Aryl Hydrocarbon Receptor
AP:	Action Potential
APD:	Action Potential Duration
BNP:	B-Type Natriuretic Peptides
BrS:	Brugada Syndrome
CABG:	Coronary artery bypass graft
CAC:	Coronary Artery Calcium S/3/ core
CAD:	Coronary Artery Disease
CAMP:	Cyclic Adenosine 3′,5′-Monophosphate
CCD:	Cardiac Conduction Disorder
CDNA:	Complementary DNA
CHD:	Coronary Heart Disease
CHO:	Chinese Hamster Ovary Cells
CK:	Creatine Kinase
CNBD:	Cyclic Nucleotide Binding Domain
CNBHD:	Cyclic nucleotide-binding homology domain
CPVT:	Catecholaminergic Polymorphic Ventricular Tachycardia
CRP:	C-Reactive Protein
CVD:	Cardiovascular Disease
CVP:	Central Venous Pressure
CXR:	Chest X-Ray
DCM:	Dilated Cardiomyopathy
EAD:	Early After/Depolarization
EAG:	Either-A-Gogo
ECG:	Electrocardiogram
EKG:	Electrocardiogram
ER:	Endoplasmic Reticulum
EtBr:	Ethidium Bromide
GBSS:	Gey's balanced salt solution

GRS: Genetic Risk Score

GWAS: Genome-Wide Association Studies

HDL: High-Density Lipoproteins

HDL-c: High-Density Lipoprotein cholesterol

hEAG: Human Ether-A-Go-go

hERG: Human Ether-À-Go-Go-Related Gene

hi-PSC-CM: Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

HF: Heart Failure

HR: Heart Rate (number of heart beats per minute)

hsCRP: High Sensitivity CRP

ICM: Ischemia

IHD: Ischemic Heart Disease

IhERG: hERG Channel Current

IKr: Rapidly Activated Delayed Rectifier Potassium Current

IKs: Slowly Activated Delayed Rectifier Potassium Current

KCNH2: Potassium Voltage-Gated Channel Subfamily H Member 2

LAD: Left Anterior Descending Artery

LDL: Low-Density Lipoprotein

LDL-c: Low-Density Lipoprotein cholesterol

LDLR: Low-Density Lipoprotein Cholesterol Receptor

LFT: Liver Function Tests

LpL: Lipoprotein lipase

LQTS: Long QT Syndrome

LQT1: Long QT Syndrome Type 1

LQT2: Long QT Syndrome Type 2

LVH: Left Ventricular Hypertrophy

MAF: Minor Allele Frequency

MI: Myocardial Infarction

MR: Mitral Regurgitation

mRNA: Messenger RNA

NA: Nucleic Acids

NEUH: Near East University Hospital

NSTEMI: Non-ST Elevation MI

PA:	Pulmonary Artery
PAS:	Per-Arnt-Sim Domain
PAH:	Pulmonary Arterial Hypertension
PBS:	Phosphate-buffered saline
PCI:	Percutaneous coronary intervention
PCR:	Polymerase Chain Reaction
PCSK9:	Proprotein convertase subtilisin/kexin type 9
PK:	Proteinase K
RCT:	Randomised Control Trial
RT-PCR:	Reverse Transcription Polymerase Chain Reaction
SAGE:	Serial Analysis of Gene Expression
SIHD:	Stable Ischemic Heart Disease
SNP:	Single Nucleotide Polymorphism
SQTS:	Short QT Syndrome
SCD:	Sudden Cardiac Death
STEMI:	ST-Elevation MI
TdP:	Torsade de Pointes
UCA:	Unexplained Cardiac Arrest
UV-Vis:	Ultraviolet Visualization
VF:	Ventricular Fibrillation
VT:	Ventricular Tachycardia
QTc:	Corrected QT
QT/QS:	Pulmonary Shunt Fraction
qPCR:	Quantitative Polymerase Chain Reaction
JWS:	J-Wave Syndrome

CHAPTER I

Introduction

1.1. An Overview of Coronary Artery Disease

The accumulation of atherosclerotic plaque in the arterial lumen is the cause of the most common cardiac disease known as coronary artery disease. This normally leads to less blood flow and decreases the capacity of the myocardium to absorb oxygen. Across the globe, this condition is one of the primary contributors of developing heart failure and mortality.

The onset of CAD is caused by various factors. These factors can be separated into two main categories: modifiable and non-modifiable. Certain attributes like age, gender, genetics, and family history, cannot be changed. Risk factors that can be changed include smoking, obesity, cholesterol, and psychosocial factors.

In the last five decades, there has been a small headway in our understanding of the genetic aetiology of CAD. It is sensible to start the process of studying the genetic factors underlying prevalent medical conditions by identifying candidate genes that code for proproteins with recognized biological importance in a disease process. Common SNPs within a gene and its associated regulatory areas can be genotyped to examine groups of affected and healthy individuals.

Over the span of minutes to hours, ion channels are constantly trafficked and internalised, controlling cardiac electrophysiology. Kv11.1, or *hERG*, drives the rapidly activating delayed-rectifier K+ current (IKr), that is necessary for cardiac ventricular repolarization. We must first comprehend the operational mechanisms of these ion trafficking channels and determine the relationship between them and CAD to achieve a more comprehensive understanding of the pathophysiology of CAD.

1.1.2. The Importance of Understanding the Role of Human Ether-A Go-Go Gene in The Cardiovascular System

The "*human-ether-a-go-go- related gene*," or *hERG*, encodes a potassium channel subunit that is a rapidly activated delayed rectifier. Delayed rectifiers

because they are essential to the repolarization phase of the action potential in excitable cells like neurons and muscle cells, potassium channels get their name. After depolarization, these channels allow k+ ions to flow outward across the cell membrane, aiding in the restoration of the cell's resting membrane potential. The ability of these channels to rectify or regulate the flow of ions in a certain direction is referred to as a rectifier. The primary function of delayed rectifier potassium channels is to permit the outflow of K+ ions from the cell, which causes repolarization. They can be distinguished from other types of potassium channels, such as inward rectifier potassium channels, which mainly permit potassium ions to enter cells, by their rectification characteristic. "Delayed" in the name alludes to these channels' distinctive time-dependent activation and deactivation dynamics.

Generally speaking, delayed rectifier potassium channels activate relatively slowly during depolarization and deactivate similarly slowly, which keeps them open during the action potential's repolarization phase. The extended repolarization phase, which is necessary for appropriate cell signalling and the general control of electrical activity in excitable tissue, is facilitated by this delayed activation and deactivation. Kv11.1, a voltage-activated potassium channel subunit, is encoded by *hERG*1. A deeper comprehension of the composition and function of this gene will aid in the understanding of CAD. Numerous studies have been conducted in an attempt to establish a correlation between a drug's in vivo tendency to elicit EADs and "torsade de pointes" and its inhibitory effect at the level of HERG K+ channels in vitro, due to the crucial part that these channels serve in the regulation of cardiac repolarization.

Problem Statement

Coronary artery disease is one of the primary contributors to mortality around the globe. There is no noticeable difference in the prevalence rates of CAD between developing and developed countries. According to one study, CAD accounts for 32.7% of cardiovascular diseases and 2.2% of all deaths globally. Due to the complexity of CAD, there have been many genes correlated with CAD none of which showed promising results. Thus far there hasn't been a single gene identified for the causation of this disease. However, *hERG* has shown proven correlations to other cardiovascular diseases including LQTS as well as ischemic heart disease. That's why it is the perfect candidate gene for this research.

Purpose of the Study

This research focuses on identifying the impact and role of Kv11.1 ion channels in coronary artery disease patients by analysing the variations in the gene expression levels of *hERG1a* and *hERG1b* in CAD patients and comparing it to those of normal individuals. This will elucidate the significance of the part ion channels play in CAD. It will also open a new path for targeted therapy and change the way diagnosis and treatments are applied today.

Research Question/Hypothesis

The main hypothesis of this research is that there is a correlation between the expression levels of hERG gene, coronary artery disease, and Long QT Syndrome. The mechanism in which alterations in *hERG* genes expression levels affect the cardiovascular system beyond prolonging the QT interval and hence causing LQTS is yet not fully understood. However, *hERG* shows a promising drug target in case a clear correlation was made between CAD and *hERG.*

Significance of the Study in Advancing our Knowledge of the Role of Voltage Gated Ion Channels in Coronary Artery Disease

Kv11.1 potassium channels, also known as EAG related gene channels, are most well-known for their role in the heart. These channels are involved in the repolarization of the cardiac action potential. Genetic mutations or side effects of certain medications that inhibit Kv11.1 currents can lead to prolonged QT interval on an electrocardiogram, resulting in long QT syndrome, a potentially life-threatening heart rhythm disorder. Kv11.1 channels also play a role in cancer. Cancer cell proliferation is reduced when Kv11.1 channel expression or currents are blocked. These channels are expressed in various tissues beyond the heart, including the brain, retina, gallbladder, stomach, and intestines. Surprisingly little, meanwhile, is understood about the expression and function of Kv11.1 channels in coronary artery disease. This research will shed light on the significance of potassium ion channels in CAD and help better understand the working mechanism of these channels by analysing the gene expression levels and investigating the causes and results of these gene expression alterations.

Limitations

This research focuses on a certain population (THE TURKISH/CYPRIOT POPULATION) making my results only applicable on this population and can't be referenced or applied for other populations. Another limitation is the sample size, due to our limited resources the sample size was limited to 68 cases both patients and control. Thus, more research should be done for more evidence on the nature of the correlation between *hERG* and CAD.

This research aims to highlight the role of the human ether-a-go-go related gene in the development of CAD and whether hERG channels can be targeted for therapy. Hence, shedding light on new treatment options and providing insight into the complex nature of this disease.

One more limitation is the methodology applied in this research. This research depended mostly on real-time PCR. Although it is trusted and dependable, more accurate and specific methodologies could have been applied including but not limited to northern blot, SAGE and RNA sequencing. Moreover, the real-time PCR-determined mRNA expression levels may not accurately represent the quantities of protein synthesis occurring within the cell. This is due to post-transcriptional regulation, Temporal differences, Translational control, and Protein stability. To obtain a thorough understanding of the cellular processes and functional results, direct measurements of protein levels, activity, or localization must be combined with mRNA expression analysis, which offers insightful information on gene regulation. The overall gene expression and cellular phenotype can be more accurately represented by combining data from both mRNA and proteins.

Objectives

- To investigate hERG1a expression levels in CAD patients.
- To investigate hERG1b expression levels in CAD patients.
- To compare hERG gene expression levels between CAD patients and healthy individuals.

Definition of Terms

Cardiovascular diseases refer to a group of disorders that affect the heart and blood vessels, often leading to impaired functioning of the cardiovascular system. A collection of conditions known as cardiovascular illnesses impact the heart and blood arteries, frequently impairing the cardiovascular system's ability to operate. These disorders cover a broad spectrum of maladies, such as heart failure, arrhythmias, valvular diseases, stroke, and coronary artery disease. Atherosclerosis, hypertension, genetic predispositions, lifestyle decisions, and underlying medical disorders are a few of the causes of them. Heart-related conditions can cause symptoms such as palpitations, exhaustion, shortness of breath, and chest pain. They also represent a serious threat to general health and wellbeing. Reducing the effects of cardiovascular disorders and increasing heart health require prompt diagnosis, treatment, and preventive actions. (McPherson, R., & Tybjaerg-Hansen, A. 2016).

Arrhythmias refer to abnormal heart rhythms that occur due to disruptions in the electrical signals that regulate the heartbeat. The heart may beat abnormally, too quickly (tachycardia), or too slowly (bradycardia) as a result of several problems. Numerous conditions, such as heart disease, electrolyte imbalances, drugs, stress, and specific lifestyle choices, can result in arrhythmias. Arrhythmias that are frequently encountered are atrioventricular block, ventricular tachycardia, and atrial fibrillation. Arrhythmias can cause palpitations, dizziness, dyspnea, chest discomfort, and fainting, among other symptoms. While some arrhythmias are benign, others have the potential to be fatal and result in consequences like cardiac arrest or stroke. Medical intervention may be required to control arrhythmias and restore a normal heart rhythm. Examples of medical intervention include medication, cardioversion, or the installation of a pacemaker or defibrillator (Malakar, A. K., et al 2019).

Heart failure is a chronic condition in which the heart's ability to pump blood efficiently is impaired. It happens when the heart muscles become weaker or stiffer, which results in the body receiving insufficient blood to meet its needs. cardiac valve problems, excessive blood pressure, coronary artery disease, and specific cardiac ailments are just a few of the factors that can lead to heart failure. Heart failure symptoms can include exhaustion, breathing difficulties, ankle and leg edema, an irregular or fast heartbeat, and trouble exercising. Heart failure is usually treated with lifestyle modifications, medicine to control symptoms and

underlying diseases, and in more serious situations, procedures like pacemaker installation or heart transplantation. (Severino, P., et al 2020).

Ischemic heart disease, also known as coronary artery disease, is a condition characterised by the narrowing or blockage of the coronary arteries, which supply oxygen-rich blood to the heart muscle. It happens when plaque, which is composed of fat, cholesterol, and other materials, accumulates on the inside walls of the arteries, obstructing the heart's ability to pump blood. Angina, or uncomfortable chest pain, can result from ischemic heart disease. Other symptoms include exhaustion, shortness of breath, and in extreme situations, heart attacks. High blood pressure, high cholesterol, smoking, obesity, diabetes, and a sedentary lifestyle are risk factors for ischemic heart disease. Treatment options include lifestyle adjustments to enhance heart health, medication to control symptoms and lower the risk of problems, and procedures like bypass surgery or angioplasty to restore blood flow. (Severino, P., et al 2020).

Coronary arteries are blood vessels that supply oxygenated blood to the heart muscle (myocardium). These arteries branch off from the aorta, the main artery of the body, and encircle the heart, forming a network of blood vessels. The heart muscle needs oxygen and nutrients to pump efficiently, and the coronary arteries are essential for this. The left coronary artery (LCA) and the right coronary artery (RCA) are the two primary coronary arteries. The left anterior descending artery (LAD) and the left circumflex artery (LCx) are the two main branches that arise from the left coronary artery's subsequent division. The left and front sides of the heart receive blood from the LAD, whereas the back of the heart receives blood from the LCx. The right side of the heart receives blood supply from the RCA. Plaque accumulation can constrict or obstruct the coronary arteries, reducing blood flow and possibly resulting in symptoms like angina or heart attacks.(McPherson, R., & Tybjaerg-Hansen, A. 2016).

CHAPTER II

Literature Review

Introduction

This literature review is going to combine, discuss and compare studies, research and articles investigating Coronary artery disease, LQTS and the mechanisms in which hERG gene affects these diseases.

CAD is a hereditary complex disease, which means it can be influenced by genetic variables and polymorphisms as well as environmental factors including but not limited to diet, lifestyle, and smoking. Many genes have been linked with CAD including genes responsible for cholesterol management, calcium channels, nitric oxide, as well as potassium channels which will be discussed furthermore.

Long QT Syndrome is mostly hereditary and can affect younger ages. It is defined by having a heart rhythm disorder medically presented as a long Q-T interval. Unlike CAD the genetic variables for hereditary LQTS are more known and well-studied. However, there is another form of this disease called Acquired Long QT syndrome, in which an individual develops this disease due to other cardiovascular problems one of which is CAD.

One of the well-known genes responsible for LQTS is the human ether-a-go-go-related gene (hERG)which is the coding gene for a subunit of a rapidly activated delayed rectifier K+ channel. There are two main types of K channels one being the calcium activated K channels and the other is voltage activated. hERG encodes for a subunit one of the voltages activated K+ channels known as Kv11.1. The mechanisms and importance of this channel will be discussed later.

This review will combine past research investigating hERG, LQTS, and CAD, to summarise the finding and wrap up everything discovered in these fields in one paper. Inspite shedding the light on many correlations between hERG and cardiovascular diseases as well as the importance of potassium channels in balancing the cardiovascular system, almost none of the articles addressed a direct relationship between CAD and hERG expression.

Coronary artery disease

CAD is a fatal cardiovascular condition that can be extremely perilous. 18.2 million adults die from it, which is nearly 6.7% of the world's population. Patients with CAD disease suffer from it as a result of malnourishment, stress, obesity, improper food, and irregular exercise. The primary goal of the clinical studies is to provide a treatment for patients suffering from this condition and provide preventative measures as well. Their primary purpose is to minimise the severity of the illness and its symptoms.

CAD is a broad condition that affects many other illnesses and increases many body risk factors. It causes progressive death by depositing cholesterol in the heart's arteries. The blood flow in arteries is impacted by cholesterol deposition, which causes atherosclerosis, which damages heart muscle. Plaque consists of fatty substances, waste materials, cholesterol, and the hormone fibrin that causes clots. This encompasses both stable and unstable angina. abrupt cardiac arrest and myocardial infarction (Figure 1).

The risk factor for CAD rises as a result of cholesterol molecules being stored or deposited. The arteries that continuously feed the heart with blood for pumping action are known as the coronary arteries. They are located right above the heart muscle.

The condition known as "ischemia" occurs when the heart lacks sufficient oxygen supply and minerals due to plaque. Chest pain brought on by ischemia are referred to as "Angina" situations.

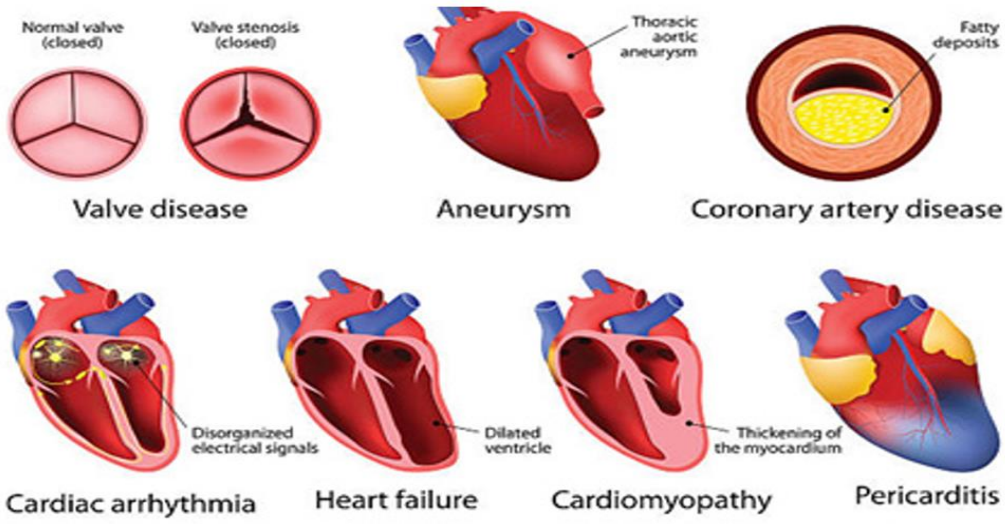

Figure 1: Visualisation of different cardiovascular diseases (Malakar, A. K.et al 2019)

Aetiology

Insufficient blood and O2 delivery to the myocardium is a defining feature of coronary artery disease (Malakar, et al (2019). Coronary artery blockage leads to a mismatch or disproportion between the heart's oxygen supply and oxygen demand, the usual cause is plaques that prevent normal blood flow in the lumen of the coronary artery.

Pathophysiology

As mentioned before, the primary differentiating physiological feature of CAD is atherosclerotic plaque buildup UI the coronary artery. This plaque consists of fatty materials that cluster inside the inner wall (also called lumen) of the vessel, narrowing it and obstructing the flow of blood. The development of atherosclerosis begins with the formation of a "fatty streak". This occurs when macrophages filled with lipids (foam cells) deposit beneath the endothelial layer, creating a fatty streak in the blood vessel. Normally, vascular injury would rupture the intima layer, allowing monocytes to reach the subendothelial area and transform into macrophages. These macrophages then absorb oxidised LDL particles, turning into foam cells. The pathogenic process is further facilitated by cytokines secreted from activated T cells. Additionally, the release of growth factors causes smooth muscle cells to produce more foam cells. The accumulation of these fatty deposits and foam cells is the start of the atherosclerotic process that can ultimately lead to blocked arteries and cardiovascular disease. This is how subendothelial plaque is created (Figure 2).

After the absorption of LDL molecules by macrophages foam cells are produced. Only T cells that are active can release certain chemicals called cytokines that help in the pathogenic phenomenon. Growth hormones cause smooth muscles to generate more foam cells, which then gather oxidised LDL particles and collagen and deposit them in close proximity to actively phagocytizing macrophages. Subendothelial plaque develops as a result of this procedure.

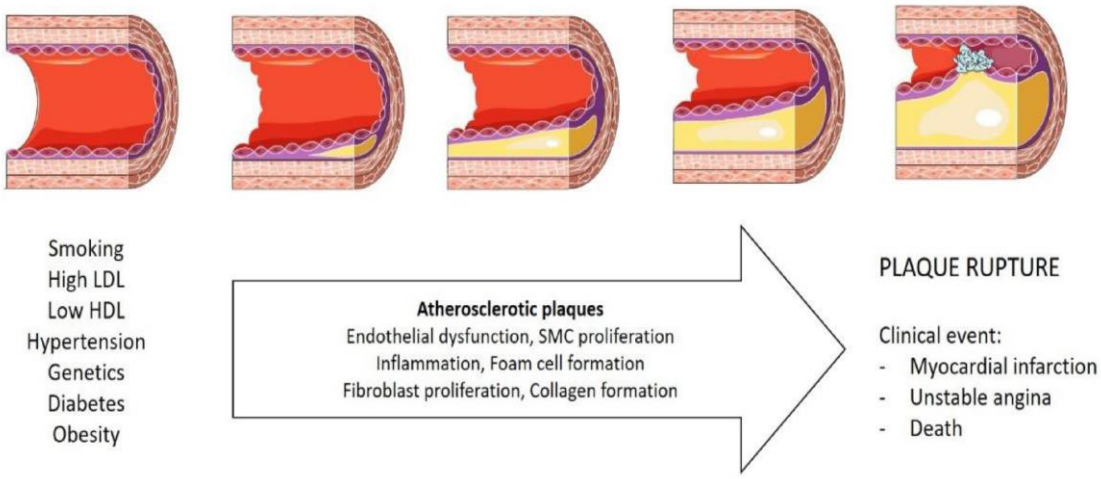

Figure 2: Plaque formation and progression in the coronary arteries (Conceição, G.et al 2020)

Two of the possible markers of CAD are Acute coronary syndrome (ACS) and stable ischemic heart disease. If not attended to immediately, could result in congestive heart failure.

Diagnosis

Several basic modalities, such as blood tests, cardiac catheterization, stress testing, Echo, CXR, and EKG, are used to screen for coronary artery disease. The conditions that the patients are presenting with are taken into account when doing these tests (Severino, et al 2020).

The electrocardiogram (EKG) is an extremely simple yet highly valuable test for identifying coronary artery disease. It involves attaching ten electrodes or "leads" to the patient's skin, which then record the electrical activity of the heart at specific points in order to measure the electrical activity in the cardiac system. It provides information on the structure and operation of the heart. When the test is over, a paper is usually generated with 12 leads, each of which represents a different spot on the heart. The heart's axis, pace as well as rhythm are crucial parameters to observe on an EKG. Thereafter, specifics about both acute and chronic pathologic processes are accessible. Acute coronary syndrome is characterised by deviations in the ST segment and sometimes the T wave. When the Acute Coronary Syndrome is shown to has progressed into cardiac arrhythmia, this is also evident. Details such bundle branch blockages, ventricular hypertrophy, and axis deviation might be found on an EKG in a chronic disease.

Echocardiography is a type of cardiac ultrasound imaging that is considered a useful and non-invasive technique for testing for various cardiovascular diseases. It can provide detailed information about the size of the heart chambers, as well as detect signs of viral or autoimmune disorders, valve regurgitation and stenosis, and abnormalities in heart wall motion. Furthermore, it aids in the identification of acute lung conditions. It also assesses the area surrounding the heart. It may be used to track these data as well as a response to the therapy in long-term situations. In an outpatient setting, it is also used as a part of stress testing. It plays a part in treatments as well as diagnostics. For instance, echocardiogram can be used to guide the needle during pericardiocentesis. In contrast to an EKG, this test is dependent on the user and may be expensive.

Stress Test The stress test is another non-invasive technique to test for CAD. In patients exhibiting angina and/or angina-like symptoms, it can help rule out or confirm heart illness when assessed carefully. An artificial stress test is performed on the heart; if abnormal alterations in ST segments of the EKG or symptoms of angina are experienced by patient, the operation is terminated, and CAD is established. Electrocardiograms are performed prior to, throughout, as well as following the procedure, the individual is also kept under observation for any symptoms. The main categories of stress evaluations are pharmacological stress tests and exercise stress tests. During exercise stress testing, the person is expected to run on the treadmill until they reach 85% of their predicted maximum heart rate based on their age. Hence, CAD is verified or denied in the event that a patient exhibits ventricular or supraventricular arrhythmias, exertional hypotension, hypertension, or any alterations in the ST-segment.

Blood Work The detection of blood products aids in diagnostic and treatment efficacy assessment. Along with the need to complete blood counts and metabolic panels, tests for cardiac enzymes and B-type natriuretic peptides are frequently performed in acute conditions. BNP can provide details about excess volume of cardiogenic origin, notwithstanding its limitations. When it comes to obesity and renal diseases, it can seem artificially elevated and misleadingly low. Two cardiac enzymes that provide information regarding an acute ischemia episode

are troponin and CK. In chronic circumstances, lipid panels provide important predictive information. Hemochromatosis is one invasive procedure that can affect both the liver as well as the heart simultaneously.

Cardiac catheterization is considered the most accurate and reliable procedure for diagnosing ischemic coronary heart disease. However, it is an invasive procedure that carries certain risks. Not all patients are suitable candidates for this invasive testing. Individuals with an intermediate pre-test likelihood of having coronary artery disease are typically good candidates for cardiac catheterization, especially in non-emergency situations where there is no acute coronary syndrome present. While cardiac catheterization provides very precise diagnostic information, the invasive nature means it is not appropriate or recommended for every patient suspected of having coronary heart disease. All STEMI patients and certain NSTEMI patients receive immediate cardiac catheterization in the event of an acute coronary syndrome. This is a skilled procedure that is carried out at a cardiac catheterization facility under mild anaesthesia. Due to contrast exposure, there is a chance of serious allergic reactions and kidney damage during the procedure.

Acute Coronary Syndrome

The hallmark of acute coronary syndrome is sudden chest pressure or pain, which usually extends to the left arm and neck. Additional symptoms that could be present with this illness include syncope, palpitations, dyspnea, dizziness, or recently developed congestive heart failure.

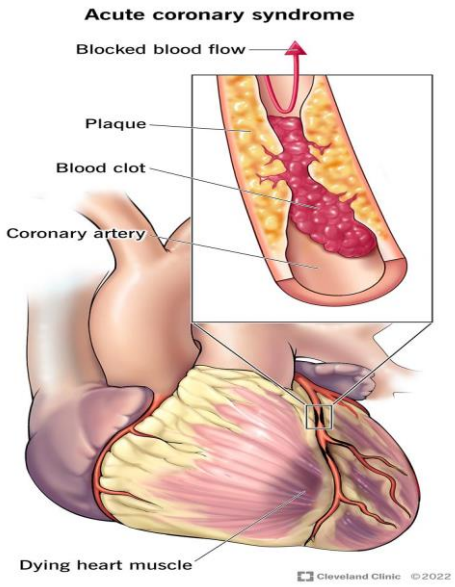

Figure 3: Illustration of the acute coronary syndrome pathophysiology (Cleveland Clinic, 2022)

Differential Diagnosis

Due in large part to the heart's close proximity to surrounding organs like the lungs, main arteries, stomach and specific bones and muscles, there is a wide range of differential diagnoses for CAD. Acute anginal chest pain can be caused by a number of illnesses, including pneumonia, pleural effusion, peptic ulcer disease, acute pericarditis, myocarditis, acute bronchitis, and others. To focus the differential diagnosis and pinpoint a precise diagnosis, the history, physical examination, and diagnostic procedures must be carefully completed (figure 3).

Toxicity and Adverse Effect Management

The treatment of ischemic coronary heart disease with both medicine and surgery has drawbacks and consequences. Patient education, physician skill, and careful selection could all help to lessen these negative consequences. Drug responses that are allergic, idiosyncratic, and cause bleeding are linked to aspirin therapy. Among the side effects of statin medication are arthralgias, myalgias, and diarrhoea. Hypotension and bradycardia may be brought on by beta-blockers. Hypotension, vertigo, elevated creatinine, coughing, and allergic responses, including angioedema, are possible side effects of ACEIs. PCI may result in stent thrombosis in an emergency situation, chronic in-stent restenosis, and coronary

artery perforation, infection, phrenic nerve damage, post-operative haemorrhage, arrhythmias, cardiac tamponade, and renal impairment are just a few of the risks that might arise with CABG.

Risk factors

Modifiable and non-modifiable risk factors fall into two groups when it comes to coronary artery disease. We start by discussing risk factors that are unchangeable:

Age: After the age of 35, CAD is more common in both men and women.

Gender: Males are more vulnerable than females.

Family history: The history of one's family is another significant risk factor. An increased risk of dying from CAD exists in people under 50 years old with a family history of early heart ailment.

The role of modifiable risk factors is smaller, but not insignificant. Only two-thirds of patients, however, get the most effective pharmaceutical treatments available. If this were to happen, CAD incidences would significantly decline. One study found that the best risk factor profiles were considerably less likely to die from cardiovascular events.

Hypertension: Approximately one in three people experience high blood pressure. The two leading causes of death in 2009 research evaluating twelve modifiable risk variables were smoking and hypertension. However, only more than half of these people manage their blood pressure appropriately.

Hypertension has been widely accepted as a major risk factor for cardiovascular diseases as a result of the amount of oxidative and mechanical stress it places on the arterial wall.

According to a 1996 publication, there was a 20-mmHg systolic and 10 mmHg diastolic increase in the Framingham cohort between the ages of 30 and 65.

Hyperlipidaemia: This condition is regarded as the second most prevalent risk factor for myocardial infarction.

An investigation using the coronary calcium score found that the prevalence of low HDL-c, combination hyperlipidaemia, and hypercholesterolemia was, respectively, 55%, 41%, and 20% higher. There is evidence that increases in

triglycerides are correlated with CAD, although the association is more complex and less obvious when additional risk factors like insulin resistance, and poor diet are considered. As a result, it is challenging to pinpoint any one mechanism via which triglycerides influence CAD.

Genetics

Significant hereditary components of CAD and environmental influences are thought to be similar. Based on family and twin studies, It has been estimated that between 40% and 60% of CAD cases are heritable; this method produces results with excellent precision even in the presence of bias. After accounting for established risk factors for coronary artery disease (CAD), the Framingham Offspring Study found that participants with a family history of early-onset CAD had more than double the age-specific incidence of developing CAD, compared to those without a family history. This is consistent with recent findings indicating a significant hereditary component to early-onset CAD occurrences.

As mentioned before, it is sensible to start the process of studying the genetics of complex diseases by identifying candidate genes that code for proproteins with recognized biological importance in a disease process (McPherson & Tybjaerg-Hansen, 2016). Common single-nucleotide polymorphisms within the gene can be genotyped to examine groups of affected and healthy individuals.

There is currently no evidence that single-gene illnesses are associated with either plaque rupture or coronary atherosclerosis, except uncommon single gene lipid metabolic disorders like familial hypercholesterolemia. A 53-kb LD block containing numerous strongly related SNPs was discovered in the 9p21.3 locus in 2007 by three distinct groups. This was the first trustworthy connection that was found for CAD by the GWAS technique.

It highlights the possibility of discovering that the risk loci identified by the GWAS approach contain genes that play a role in lipoprotein metabolism, hypertension, and other symptoms related with coronary artery disease. However, several unique regions harbouring genes that are not well understood in relation to atherosclerosis and plaque rupture are very important.

Merely 11% of the estimated heritability of coronary artery disease was justified by the 48 GWAS significant loci that had been previously discovered. An

approximate joint association research employing genome-wide complex trait analysis software found 202 false discovery rate variations in 129 loci. As a result, there were multiple independent indicators of CAD connection at multiple loci. When combined, the 202 independent variants explained around 28% of the predicted heritability of CAD. Of these, fifteen only made up 2% of the heritability of CAD and had low frequency (MAF<0.05). Overall, this investigation has added to our knowledge of the genetic makeup of CAD and has strengthened the theory that genetic vulnerability to coronary artery disease mostly resulted from the limited impact size of several common SNPs. A discovery rate of q<0.05 revealed independent enrichment for DNase I hypersensitive sites and histone/chromatin alterations across 11 cell types, which is consistent with earlier findings showing GWAS signals are enriched in genomic regulatory areas.

Numerous biomarkers discovered through epidemiological research have been connected to CAD. While observational epidemiological studies can provide valuable insights, two major limitations are the issues of confounding and reverse causality. These restrictions inhibit the kind of study that may be used to prove a cause-and-effect relationship between a biomarker and a risk factor. An increasing number of epidemiologists are using the Mendelian randomization (MR) method, which is similar to the randomised controlled trial (RCT), to avoid confounding and reverse causality. Most misinterpretation and reverse causation can be avoided by looking at common genetic variations associated.

One of the key MR constraints that RCTs share is pleiotropy, or the need that the genetic variant(s) in question only affect the pertinent biomarker. This isn't always true, though, because pleiotropic effects have been shown for a number of genes, including APOA5, CETP, and LPL, which affect plasma lipid characteristics.

There is no proof that high density lipoprotein (HDL) cholesterol and C-reactive protein (CRP) cause heart attacks, despite the fact that they are risk factors. Utilising MR to ascertain the causative links between plasma levels of residual cholesterol (triglycerides), lipoprotein(a), and LDL-C and the risk of CAD has made significant progress.

Genetic variations, including common copy number variations and single nucleotide polymorphisms (SNPs), in the LPA gene are associated with elevated

levels of lipoprotein(a). These genetic factors that increase lipoprotein(a) have also been linked to an enhanced risk of coronary artery disease (CAD) and aortic valve stenosis.

Because of mutations in the LDLR, familial hypercholesterolemia shows that raised LDL-C levels are directly linked to an increased risk of CAD. Certain correlations between plasma LDL-C concentrations and genetic variations in the PCSK9, NPC1L1, and HMGCR loci are linked to an elevated risk of CAD. Mutations in the PCSK9 gene that increase its function are the cause of autosomal dominant familial hypercholesterolemia, as first reported by Abifadel et al. Subsequently, it was demonstrated by Cohen et al. that PCSK9 loss-of-function mutations were linked to decreased lipoprotein C plasma concentrations and a decreased incidence of CAD.

With this finding, PCSK9 became a promising new target to reduce lipoprotein C levels. By inhibiting the NPC1L1, which is a transporter protein that is responsible for absorbing cholesterol from the intestine into intestinal cells, and from bile into liver cells, ezetimibe lowers LDL-C levels.

Ezetimibe has lately been demonstrated to lower cardiovascular risk in spite of this. Researchers from the Myocardial Infarction Genetics Consortium studied the NPC1L1 gene by sequencing it. in 14,728 controls and 7364 CAD patients and found 34 mutations that rendered the protein nonfunctional. In a much larger replication research, one of these, p.Arg406X, was linked to a 50% reduction in the risk of CAD and a 10% decrease in LDL-C.

In contrast, Lauridsen et al. included 67,385 participants in single-centre research of the general community; of these, 5255 and 3886, respectively, had experienced ischemic vascular disease (such as heart attacks or strokes). It was demonstrated that these differences were linked to stepwise lifetime decreases in LDL-C levels of ≤3.5%, which in turn were linked to a decline in cardiovascular diseases risk. This was achieved by employing a genetic score of frequent variants in NPC1L1 to simulate the effects of ezetimibe. Thus, the study hypothesised that a higher risk of symptomatic gallstone disease would be a longer-term, biologically reasonable side effect of ezetimibe medication.

Lastly, Ference et al found that shared genetic polymorphisms linked to NPC1L1 and HMGCR both resulted in a decrease in LDL-C and a corresponding lower CAD risk in research involving 108 376 participants in 14 clinical trials. As a

result of these studies, there is strong evidence linking the concentrations of LDL-C plasma to CAD. This is due to the fact that the three aforementioned genes all modify LDL levels independently of other lipid or nonlipid factors. Notably, variations in the LDL-C or the impact of LDL-C decrease in statins (RCTs) are not as significantly correlated with CAD as variances in the LDL-C concentration-related genes PCSK9 and NPC1L.

This is most likely due to the fact that hereditary influences include lifetime exposure to LDL-C. The role of plasma triglycerides as a stand-alone risk factor for CAD has been a point of discussion for a long time. However, genetic studies have provided evidence in favour of the idea that lipoproteins rich in triglycerides, or their residues, are the cause of atherosclerosis. The main modulator of plasma triglyceride metabolism is lipoprotein lipase. The risk of developing heart and cardiovascular disease, as well as the risk of death from any cause is increased by genetic polymorphisms that reduce LPL activity.

ApoA5 stimulates LPL activity, whereas ApoC3 suppresses it. The incidence of coronary heart disease and plasma triglyceride levels are linked to mutations in APOC3, but APOA5 loss-of-function mutations had the opposite effect. Genotype combinations of three frequent variants in APOA5 were associated with 1.87 odds ratios for myocardial infarction and increases in estimated remnant cholesterol and triglycerides, according to Jørgensen et al.

Even though the effects of each found variation in CAD susceptibility are small on their own, these effects are cumulative and independent. These can be added to an individual's GRS, which is the overall total of risk alleles modified for each one's unique effect magnitude. The idea that the genetic predisposition or risk for developing coronary artery disease (CAD) is primarily obtained from the influence of numerous SNPs with minimal impact sizes is well supported overall by the most current GWAS data. Previous studies that only partially sequenced likely genomic areas in CAD kindreds discovered a number of uncommon genetic variations, such as mutations or changes in gene copy numbers, that are linked to an increased risk of coronary artery disease (CAD); nevertheless, replication failed in the majority of these cases.

It's also possible that a sizable number of more prevalent SNPs are associated with CAD but haven't shown significance in GWAS due to tiny impact sizes, low allele frequencies, or inadequate sample sizes. However, genetic variants that only

show a small or weak association may still provide valuable information about the molecular causes of the condition especially if those weakly linked variants are found to be clustered within a common functional group or biological pathway. This is based on the hypothesis that clinically significant polymorphisms linked to complicated illness are hidden within networks of closely interacting genes. In light of underlying biological processes and functional relationships between genes, genomic data analysis could offer new perspectives or understanding into the genetic architecture of CAD.

Even with GWASs's recent success, about 80% of CAD's estimated heritability is still unknown. Gene environment interaction, in which the influence of a specific a genetic variant may only have an observable effect or impact when it interacts with another genetic or environmental factor that modifies its expression, like obesity and smoking, may account for part of this missing heritability. The investigation of the molecular pathways that underpin the intricate construction of complex illnesses is another application of the route approach. The interactions between many components, including as proteins, genes, and metabolites, form the foundation of a molecular network. These could include a variety of functional connections, coexpression, impacts on the regulation of genes, and protein interactions. Genes connected by physical or functional modules usually reside in similar molecular networks and are linked to the same or related illnesses. Thus, studying molecular networks offers more insights into the connections between specific groups or subsets of genes that are part of a common biological pathway as well as significant interactions between the constituents of other biological pathways.

Long QT Syndrome

As mentioned before Long QT Syndrome is defined by an abnormality in the heart's electrical recharging system. Unlike other cardiovascular diseases it does not affect the structure of the myocardium.

The heart's electrical system powers the contraction and relaxation of the heart chambers instructing them when to pump blood.

This electrical system consists of electrical signals also known as impulses that are usually directed from the top of the heart to its bottom. Each impulse induces one heartbeat after which the electrical system needs time to recharge before the next beat.

In LQTS the time needed to recharge between following beats is usually longer. This delay is what is known in medical terms as prolonged QT interval. This usually is observed in an electrocardiogram (EKG).

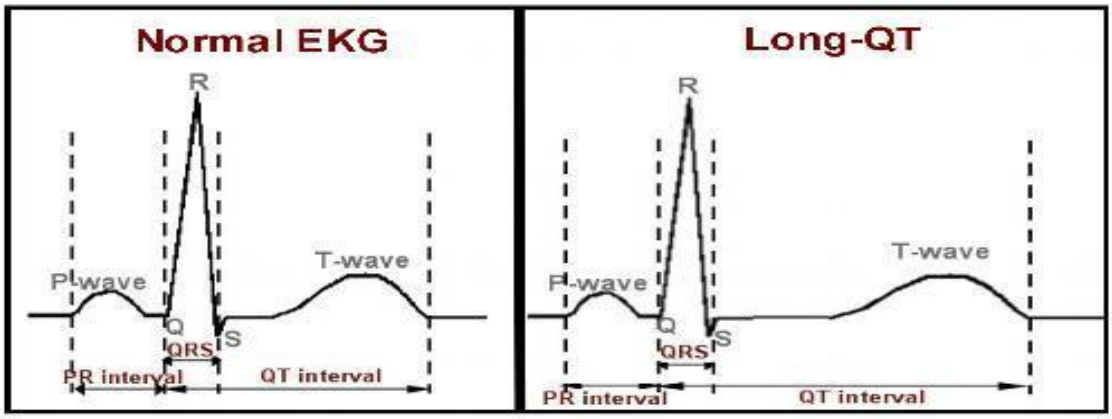

Figure 4: Electrocardiograms showing the difference between the QT- intervals of healthy versus LQTS patients (Treatment for Long QT Syndrome (LQTS) in Washington DC & Maryland, n.d.)

LQTS can be categorised into two main domains depending on their origin, congenital LQTS and acquired LQTS. Congenital LQTS is hereditary and accounts for more than half the LQTS cases. Many genes were correlated with congenital LQTS including but not limited to hERG. Congenital LQTS can occur in two forms. The first one is autosomal dominant known as Romano-Ward Syndrome which is more common in individuals where one gene variant is passed down from a parent.

The other form is called Jervell and Lange-Nielsen Syndrome which have an autosomal recessive mode of inheritance. In this case the LQTS is more severe as the individual inherits copies of the variant gene from both parents. This syndrome appears earlier in life where children are born with LQTS and it is usually accompanied by deafness.

Whereas the acquired form of LQTS is usually caused by environmental factors. For Instance, tens of common medications such as certain antibiotics (erythromycin, Zithromax), antiarrhythmic and antidepressants can be a trigger for prolonged QT intervals in healthy individuals. This case is known as Drug Induced Long QT Syndrome.

Besides drugs, some other conditions can be responsible for acquired LQTS including but not limited to hypothermia, hypomagnesemia, hypokalaemia, intracranial bleed and hypothyroidism. Acquired LQTS usually can be reversed by treating the cause.

The key features of the inherited long-QT syndrome are an extended time period for the electrical activity in the ventricles of the heart to return to its resting state, a wide range of clinical symptoms including fainting episodes related to abnormal heart rhythms, and the potential for sudden unexpected death (Figure 4). The LQT2 variety of LQTS is caused by mutations in hERG. Studies on cellular expression have linked these mutations to a reduction in the delayed rectifier repolarizing current's fast component (IKr). A study by Arthur J. Moss et al at the University of Rochester Medical Canter, examined the prognostic significance and clinical characteristics of hERG channel pore and nonpolar region mutations in the LQT2 variant of this illness (Moss, et al 2002). 201 patients had a total of 44 distinct hERG mutations found in them, 14 of which were in the pore area. There were 166 non-pore region mutations and 35 pore region mutations in the individuals. Follow-up continued until the age of 40. In comparison to participants without pore mutations, Pore mutation carriers experienced more severe physical symptoms and a higher rate of cardiac-related events associated with arrhythmias that happened earlier in life. Pore mutations were found to be the main cause of risk. For QTc at 500 ms, the hazard ratio ranged from 11 (P 0.0001) to 16, with the pore hazard ratio increasing by 16% for every 10 ms rise in QTc. Compared to individuals without pore mutations, patients with hERG gene pore region mutations have more chance of developing cardiac events due to arrhythmias.

Clinical aspects of LQT2

An abnormal prolonged QT interval on an electrocardiogram is used to identify and confirm long QT interval chronic QTS. Unknown seizures or syncope are frequent indicators of LQTS. How long it will take for the ventricles to

electrically repolarize is indicated by the QT interval. The characteristic EKG waveform with LQT2 is a notched T-wave plus a prolonged QT interval. However, not everyone who has had their LQT2 gene validated exhibits this trait.

Torsade de pointes, also referred to as "twisting of the points," is a particular type of ventricular tachyarrhythmia that causes syncope in relation to long QT syndrome. One obvious indicator of it is a wave-like or sinusoidal pattern in the angle or orientation of the QRS complex (which represents ventricular contraction) around the baseline or isoelectric line on the electrocardiogram trace. Ventricular fibrillation can arise from torsade de pointes or terminate on its own. Ventricular fibrillation is a severely disordered electrical activity and a leading factor of mortality. Although the precise frequency of congenital LQTS is unknown, estimates of its prevalence range greatly. Most frequently, dominant mutations in KCNH2 or KCNQ1 result in congenital LQTS. Most individuals with long QT syndrome have anatomically normal hearts.

Long-term heart failure and medication side effects that prevent hERG1 channels from moving from the ER to the cell surface can also cause LQTS. In addition, gender, hypokalaemia, and bradycardia are important risk factors for either acquired or congenital long QT syndrome. It should come as no surprise that individuals with congenital LQTS are probably significantly more prone to experience drug-induced arrhythmia, regardless of the specific genetic change or mutation involved, although it has been challenging to definitively confirm this relationship. Reduced ability to convert drugs to a hERG1-inactive metabolite because of illness or alteration in particular CYP isoforms is another significant risk factor. Moreover, Coad Administered medications like terfenadine and cisapride cannot be converted to their hERG1 inactive metabolites by CYP3A4 inhibitors (such as erythromycin and other macrolide antibiotic therapies). Drug-induced QT prolonging, like LQT2, can result in major adverse effects, such as torsade de pointes arrhythmia, ventricular fibrillation, and unexpected fatality

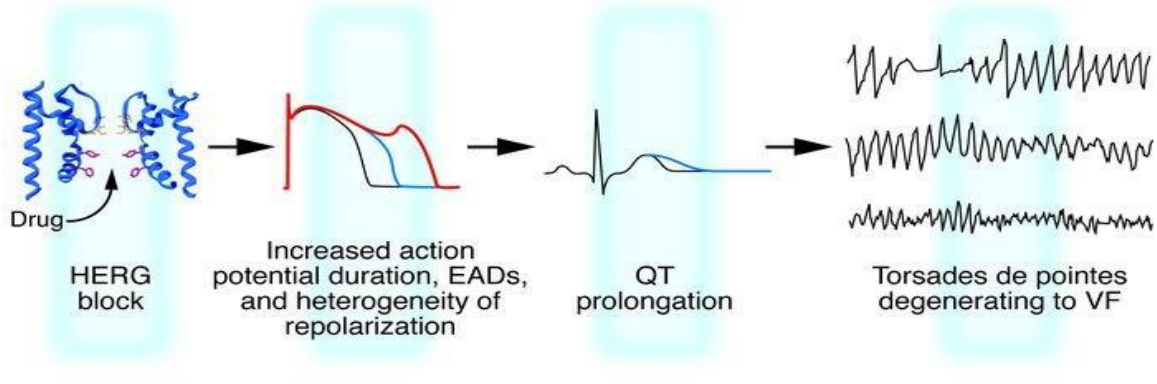

Figure 5: Mechanism in which hERG channel dysfunction leads to acquired LQTS (Roden & Viswanathan, 2005)

There was a strong push for pharmacologically induced QT prolongation, a "class III" pharmacological action that was proved later to be an antiarrhythmic mechanism to frequently be facilitated by hERG1 channel blockage. As part of the standard drug development procedure, pharmaceutical companies actively check all noncardiac treatments for this undesirable side effect. These businesses have given up on creating medications with this action, though, because extended electrical refractoriness of the ventricle has a significant proarrhythmic potential and can prevent some types of arrhythmia.

The course of treatment for variants of other diseases is the same for LQT2. Beta-receptor blocker therapy is the most widely used type of treatment. During a thorough 5-year study, 869 LQTS patients were found to benefit from beta-blocker treatment. The probands experienced a reduction in syncope, abortive cardiac arrests, and mortality from 0.97 to 0.31 incidences yearly, whereas the affected family members experienced a decrease from 0.26 to 0.15 events annually.Activators of hERG1 channels have recently been found , however none of these substances have been tested in clinical trials to ascertain their safety or effectiveness. Left cardiac sympathetic denervation or implanted cardioverter defibrillators are common treatments for high-risk patients. In the largest research, which included 147 patients with long QTS, cardiac events were reduced by over 90% when left cardiac sympathetic denervation was applied.

hERG1 channels and the long QT syndrome

Jervell and Lange-Nielsen originally recognized the disorder now called "long QT syndrome" in 1957 in deaf-mute youngsters who also showed prolonged QT

intervals, many syncope episodes, as well as a high rate of sudden death. After a few years, patients who suffered from syncope and abrupt death but with no deafness were independently reported by Ward and Romano. This early research showed that ventricular arrhythmia linked with a longer QT interval was hereditary in at least some cases. Over the course of the next 25 years, a great deal of work was done to record the clinical manifestations and create treatment plans; Moss and his fellow researchers made the most contributions in this regard. In 1991, researchers started to investigate the underlying genetic causes of the congenital (present at birth) features of this disorder. They eventually identified a small region on chromosome 11 that was found to include the gene KvLQT1 (now called KCNQ1), which provides instructions for producing the alpha subunits that come together to form the IKs channels. The work of researchers like Keating and Vincent connected this specific gene to the long QT syndrome (LQTS) phenotype observed in a large family group.

By 1997, hereditary LQTS was associated with four distinct ion channel gene variations. Accompanying the identification of novel ion channels was a swift advancement in our understanding of the cell-level causes of LQTS and related channelopathies (Figure 5). Two more K+ channel gene subfamilies, EAG and ERG, were found in 1994 because of low stringency screening for genetic sequences that are like the eag gene, which encodes a potassium channel protein that is expressed in both fruit flies and mammals, in a human hippocampal cDNA library. Later research showed that the HERG gene (now known as KCNH2) provides the instructions for producing the alpha subunits that make up the potassium channel responsible for generating the rapid delayed rectifier potassium current, or IKr, in the heart. A year later, LQTS was linked in numerous families to mutations in the gene HERG. A prolonged QT interval is a clinical marker for twelve distinct recognized hereditary arrhythmias at this time.

A database of hereditary arrhythmias (http://www.fsm.it/cardmoc/) indicates that as of September 2009, 291 distinct hERG1 mutations were known to induce LQT2. However, it's evident from a report published in late 2009, researchers stated that they had identified an additional 159 unique genetic variations or mutations in the KCNH2 gene in a sample of 2,500 people who exhibited the long QT syndrome (LQTS) phenotype, as well as 903 individuals who tested positive for carrying one of these genetic changes. However, the researchers noted that this is just a small fraction of the total number of mutations that will eventually be found to be

responsible for causing the LQT2 subtype of long QT syndrome. This brings the total number of KCNH2 mutations associated with LQT2 to 450 and demonstrates the broad distribution of hERG1 subunit modifications.

Of the 226 genetically verified cases of LQT2, missense mutations accounted for 62% of the sample, followed by frameshift mutations at 24% and mutations in the KCNH2 gene that result in premature stop codons, disruptions to the normal splicing process, or small insertions or deletions that do not shift the reading frame at 14%. Functional, in vitro electrophysiological studies have only partially defined (and hence verified) the mutations in KCNH2 utilising a technique called whole-cell voltage clamp, researchers measure and compare the current magnitude and other electrical/physical properties of mutant ion channel proteins produced in the lab against the normal, wild-type version of the channels to identify the functional consequences of hERG1 mutations. Inside the endoplasmic reticulum (ER), the core structure of the hERG1 protein undergoes a process called glycosylation.

The ubiquitin-proteasome system then quickly breaks down the parts that are retained in the ER. Missense mutations in hERG1 are currently assumed to induce function loss mostly by the protein folding incorrectly, getting stuck and accumulating in the endoplasmic reticulum, or having problems with the normal transport and trafficking to the Golgi apparatus and the cell membrane. If it is possible to compel many of these mutant channels, one way to help the mutant protein reach the cell membrane is by lowering the temperature during the cell culture process or by employing other methods, they may still operate, albeit with altered kinetic properties, or via pharmaceutical intervention using medications that would ordinarily block the channel.

In Xenopus oocytes cultured at truncated temperatures, heterologous expression was used to conduct the first functional investigations of mutant hERG1 channels (Moss, A. J., et 2002). This led to the erroneous notion that some mutations had an impact on channel gating, which in turn reduced current amplitude, although the main flaws were actually sensitive to temperature, incorrect folding and decreased trafficking. Pentamidine and arsenic trioxide (As2O3) are two medications that are also capable of lessening hERG1 channel flow. These medications work by severing the connection between hERG1 channels and their proteins. Because of this, medications can decrease the quantity of hERG1 protein that is delivered to the cell membrane, or they can block channels directly in order to diminish hERG1 activity.

Tests have therefore been created for both mechanisms. Uncommon homozygous mutations in KCNH2 that are proven genetically cause severe QT prolongation in a live birth or intrauterine mortality. For LQT2, heterozygous hERG1 mutations are therefore frequently found. Expressing both the mutant and the normal, wild-type versions of the hERG1 protein subunits together in the same cells is necessary to evaluate the issues brought on by a particular heterozygous variation in KCNH2. This method facilitates the determination of whether a specific missense mutation reduces channel density and/or function in a dominant-negative manner. For instance, hERG1 protein subunits that contain a specific mutation, where the amino acid glycine (G) is substituted for serine (S) at position 638 in the pore region serves as a "poison pill" because they cling readily to functional hERG1 subunits and block tetrameric channels, which may hold a single mutant subunit. Arrhythmia abruptly ended the lives of more than half of the transgenic rabbits, G628S. Interestingly, in these rabbits, the fall in IKs was accompanied by a decrease in IKr, rather than the normal, expected increase in a current that helps depolarize the cell membrane; we see a different response.

Haploinsufficiency, a disorder in which only About half of the total hERG1 channels are probably either absent or not working properly (rapidly broken down), and the mutant and normal versions of the subunits do not interact with each other, can result from even certain missense mutations. This can be brought on by more disruptive changes, like frameshifts or various other disorders.

Certain gene alterations lower IKr by changing the hERG1 channel's gating mechanism, albeit they are clearly less common than folding and trafficking abnormalities, which are thought to account for up to 90% of missense variants. The outward flow of electrical current that the IKr channel helps generate during the repolarization phase of the cardiac action potential is reduced by mutations that either accelerate the pace of deactivation or encourage inactivation. Instead of shortened protein synthesis, a nonsense mutation generates nonsense-mediated mRNA degradation.

The potential that the specific positions where mutations occur within the hERG channel protein could be related to the severity of a disease was investigated in 2002 by Moss and colleagues. Of the 201 individuals with 44 distinct variants, 35 had a changed pore area. In comparison to individuals without pore mutations, patients with pore mutations (n = 14) experienced twice as many cardiac events

linked to arrhythmias and more severe clinical symptoms. Mutations that occur within the pore region of the channel are more prone to causing a dominant-negative effect, even if electrophysiological studies have not shown evidence of one. If the channels are successfully transported from the Golgi apparatus to the cell's outer membrane, this effect could be achieved by either inhibiting ion conductance or increasing the pace where the multiple subunits that assemble together to form the channel start to fall apart within the endoplasmic reticulum and Golgi apparatus.

A study conducted in China by Chuangjia Hu et al investigated the decreased expression of the human ether-a-go-go-related gene in rat models of cardiac hypertrophy since QT prolongation and cardiac abrupt death are correlated with a higher incidence of cardiac hypertrophy (Hu, et al, 2011). Using rats as a model system, Chuangjia Hu in his work investigated the expressional modulation of the rat version of hERG, which provides the instructions to make a key subunit of the fast-acting potassium current (IKr) that contributes to the repolarization of the cardiac action potential, during the development of cardiac hypertrophy. In Sprague-Dawley rats, a surgical procedure that constricts the abdominal aorta [which results in the development of left ventricular hypertrophy (LVH) in this group of animals], was used to induce cardiac hypertrophy. A control group, or Ctrl group, was defined as rats that had not had surgery. Six weeks following the procedure, measures related to hemodynamic, morphology, and histology were noted. Furthermore, a combination of Western blot, immunohistochemical analysis, and real-time PCR was utilized to assess the expression of HERG.

To sum up, the findings support a possible new mechanism for HERG regulation and, by extension, a longer QT interval during the course of LVH formation. In-depth knowledge of LQTS development would be possible through future research on the processes by which intracellular signalling and pressure overload control the production and expression of the hERG gene within the left ventricular cardiac muscle cells. This would make it easier to design therapeutic drugs that specifically target the development of LQTS in cardiac hypertrophy.

Correlation between Long QT Syndrome and CAD

Individuals with severe blockages in their coronary arteries (advanced coronary artery disease or CAD) are at a higher risk of experiencing sudden cardiac

death, which is linked to an abnormal prolongation of the QT-interval on the electrocardiogram, reduced left ventricular function, and other variables.

A study conducted by B. KRAMER at Medical University Hospital (New Krehl Clinic) in Germany shed light on the correlation between the severity of coronary artery blockages, the level of left ventricular pumping function, and the duration of the QT-interval observed on an electrocardiogram (Krämer, B., et al 1986). where QT-intervals were associated with coronary angiographic data in 304 patients who had catheterization because of suspected CAD. The ejection fraction of the ventricular angiogram was used to measure the function of the left ventricle, and coronary angiograms were categorised into 1-, 2-, and 3-vessel disease based on the Gensini score.

In the results, only individuals with reduced left ventricular function showed significant alterations in QTc among patients with 1-, 2-, and 3-vessel illness. From one to three vessel diseases, the QTc-interval dramatically increased in these patients. QTc prolongations were observed if the criteria for defining significant coronary artery stenosis (narrowing) was increased from 50% or greater to 90% or greater, the difference in corrected QT-interval duration became more pronounced in patients with single, double, or triple vessel coronary artery disease. This exaggerated effect was especially seen if the left main coronary artery or the proximal part of the left anterior descending artery (LAD) was affected. The results indicate that the presence of significant narrowing in the proximal left anterior descending (LAD) artery or the left main coronary artery, along with the involvement of all three major coronary arteries (3-vessel disease), and impaired left ventricular pumping function are among the established factors contributing to sudden death that are correlated with extension of electrical systole. However, because of a significant interindividual heterogeneity in the data, the predictive usefulness of a single QTc-determination in a single patient is restricted.

So, this study concluded an obvious relationship between CAD and Long QT syndrome. It showed that they are directly related in which as the degree of CAD increases the QT interval is prolonged.

Numerous researchers have looked into the relationship between LQTS and CAD. It has been discovered that the severity of CAD is strongly connected with the prolonged QTc interval, a feature of LQTS. Furthermore, QT interval prolongation can result from sympathetic hyperactivity in patients exhibiting symptoms of acute

coronary syndrome, suggesting a possible connection between CAD and QT prolongation. Moreover, a study with a large proportion of people with type 2 diabetes found a strong correlation between the length of the QT segment and the amount of calcified plaque in the coronary arteries (CAC), indicating a possible link between CAD and QT duration. All of these results point to a connection between CAD and LQTS, highlighting the significance of QT interval monitoring for risk assessment and treatment in CAD patients.

In a study including individuals with acute coronary syndrome, Tanta University researchers evaluated the correlation between heart rate and the degree of CAD (Shaimaa, et al 2021). Acute coronary syndrome patients were included in the retrospective study. With relation to admission heart rate and QTc interval, there was a statistically notable distinctions among the two groups. Both low AHR < 60 bpm and high AHR < 90 bpm groups showed a strong correlation with severe CAD. Syntax scores (severe coronary lesion) were primarily greater in patients with higher admission heart rates. In addition, compared to those without a prolonged QTc interval, those with a prolonged QTc interval had a higher risk of SS, and serious coronary artery disease. In conclusion, the present investigation showed that, in patients with acute coronary syndrome, Prolongation of the corrected QT-interval and the patient's heart rate at the time of hospital admission are independent factors that can predict the severity of coronary artery disease.

Angina pectoris and arrhythmia have been linked to an abnormal or unusual origin of the coronary arteries, another uncommon congenital abnormality. An uncommon hereditary arrhythmogenic illness called LQTS is characterised by a susceptibility to sudden cardiac death and potentially fatal arrhythmias. In a report, a 36-year-old patient with two uncommon abnormalities is successfully treated with β-blocker therapy.

The QT/QS2 ratio was found to be correlated with the severity of coronary artery disease observed through coronary angiography imaging in patients who underwent the procedure due to chest pain or discomfort. (S., Cuomo., et al1988). The study included 83 individuals with angiographic indications of congestive heart failure and Sixteen patients who were used as a control group, all of whom had normal results from their coronary angiograms and had preserved left ventricular pumping function. The extension of left ventricular aberrant wall motion affects the QT/QS2 ratio in individuals with coronary heart disease (CHD); a QT/QS2 value

greater than 0.99 indicates CHD and is indicative of diagnostic utility. The study reveals a connection between the angiographic degree of CAD and the QT/QS2 ratio, offering information about a possible diagnostic criterion.

The discrepancy between the longest and shortest QT intervals as determined by the normal 12-lead ECG is known as QT dispersion. It displays the heterogeneity of ventricular repolarization, which can occasionally result in ventricular arrhythmias. A key factor in the pathophysiology of prolonged QT dispersion that results in ventricular arrhythmias of the re-entrant type is myocardial ischemia. The goal of this research study was to assess the variability in the QT interval among patients with known coronary artery disease to the standard normal value and to determine the frequency of individuals with higher QT dispersion (Sadia, Mubarak, et al 2015). According to the study's findings, QT dispersion refers to the difference between the longest and shortest measurements of the QT interval recorded from different areas of the heart obtained from a conventional 12-lead ECG. It displays the heterogeneity of ventricular repolarization, which can occasionally result in ventricular arrhythmias. A key factor in the pathophysiology of prolonged QT dispersion that results in ventricular arrhythmias of the re-entrant type is myocardial ischemia. The goal of this research study was to assess the variation in the QT interval measurement among patients diagnosed with coronary artery disease to the standard normal value and to determine the frequency of individuals with higher QT dispersion.

Ion channels Function and Significance

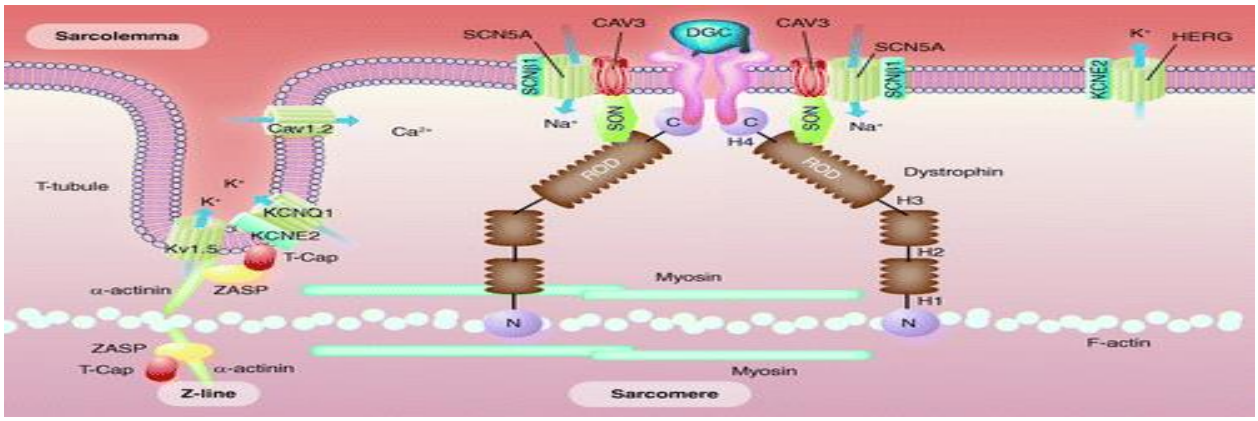

Figure 6: The ion channels mechanisms and molecular pathways (Vatta & Faulkner, 2006)

Ion channels are continuously trafficked and internalised over the course of minutes to hours, regulating cardiac electrophysiology. The rapidly activating delayed-rectifier K+ current (IKr), which is necessary for cardiac ventricular repolarization, is driven by Kv11.1, sometimes referred to as hERG. It is difficult to experimentally characterise the different temporal influences on channel trafficking and gating of genetic and acquired modulators (Figure 6).

Although there is yet no model that encompasses channel trafficking, computer models play a crucial role in explaining these effects. An experiment done by Stefan Meier et al in Netherlands introduced an innovative computer model that replicates the Kv11.1 channels' synthesis, forward trafficking, internalisation, recycling, degradation, and control by extracellular K+, pentamidine, dofetilide, and temperature, as found in experiments (Meier, S., et al 2023). In the O'Hara–Rudy human ventricular cardiomyocyte model, the trafficking model was additionally linked with the modulators' acute effects on channel gating. Kv11.1 membrane levels were significantly elevated by supraphysiological dofetilide concentrations, which also resulted in a notable channel block. Clinically meaningful concentrations, however, had no effect on trafficking.

Similar to this, long-term culture data showed that acute hypokalaemia decreased Kv11.1 membrane levels, although short-term data showed that the effect was minimal. In contrast, faster kinetics caused by clinically significant temperature increases sharply raised IKr; nevertheless, within 24 hours, IKr dropped as a result of lower Kv11.1 membrane levels.In contrast, at lower temperatures this was not the case. When combined, our model shows how temperature, hypokalaemia, and dofetilide affect channel gating and trafficking in complex ways that regulate cardiac electrophysiology over time. It also offers a framework for future research examining the contribution of heart arrhythmias due poor trafficking.

Another study was conducted by Tomas Novotny to investigate the notion that those who have survived ventricular fibrillation (VF) due to CAD have a higher frequency of ion channel gene alterations than controls. They examined the whole KCNQ1, KCNH2, and KCNE2 genes' coding sequences in 45 (five female) CAD patients who had survived confirmed VF as well as in 90 matched healthy controls (Novotny, T., et al 2011). The exons carrying uncommon coding mutations discovered survivors in the VF were sequenced in a second control group including CAD patients without malignant arrhythmias. VF survivors Had a carrier frequency

for every uncommon sequence variations that was substantially greater than that of CAD controls. Four coding variations were discovered in eight VF survivors. Three in the KCNH2 gene: P347S was linked to long QT syndrome; R148W and GAG186del are unique. P2006A variation in Five separate males were found to have the SCN5A gene. It has previously been shown that the sodium channel kinetics are barely affected by this variation. In the unaffected individuals, there were no uncommon coding variants detected. Three CAD controls contained the P2006A version. A subgroup of patients with cardiovascular condition and VF was found to have a mechanistic function for certain rare coding variations in 5 LQT genes, as evidenced by the much greater prevalence of these variants in cases compared to controls. In conclusion this study highlighted several gene mutations in CAD patients including KCNH2.

Additionally, an investigation was run by Yoshinori Nishijima and his team at Medical College of Wisconsin to better understand the contribution of specific ion channels in coronary artery disease (Nishijima, Y., et al2017). In human microcirculation, hydrogen peroxide (H2 O2) controls vascular tone in both normal and pathological circumstances. It dilates arterioles by the activation of large-conductance Ca2+-activated K+ channels in individuals with CAD; however, its exact mechanism of action differs in non-CAD participants from CAD subjects. We speculate that in non-CAD versus CAD, H2 O2-induced dilation includes distinct K+ channels, leading to a changed ability for vasodilation during illness. H2 O2-induced dilation was lessened by the selective KV 1.5 blocker diphenyl phosphine oxide-1 to a degree comparable to that of 4-aminopyridine; however, the selective KV 1.3 blocker phenyl alkyl psoralen-1 had no effect. H2 O2-induced dilatation in arterioles from CAD patients was considerably prevented by paxillin; did not inhibit this dilatation. Vascular smooth muscle fibres that were separated from CAD arterioles showed a significant reduction in diphenyl phosphine oxide-1–sensitive K+ currents and KV 1.5 cell membrane localization, whereas the expression of messenger RNA and protein remained substantially unaltered. A major contributing factor to CAD and other vascular diseases due to microvascular dysfunction may be loss of KV 1.5 vasomotor function. The findings of this study show the huge impact potassium channels have and their relationship to CAD.

Kv11.1 Potassium Channels

Kv11.1 potassium channels are necessary to repolarize the heart. Cardiac arrhythmias are brought on by prescription drugs that inhibit Kv11.1 ion channels, prolonging the ventricular AP. Remarkably little is understood about the expression and functionality of the Kv11.1 ion channel in pulmonary tissue. A study conducted by Nataliia V. Shults et al at Georgetown University Medical Center demonstrated that major pulmonary arteries (PAs) in both human and rat healthy lung tissues had high expression of Kv11.1 channels (Shults, N.,et al 2020). Rats with pulmonary arterial hypertension (PAH) and people with pulmonary hypertension linked to chronic obstructive pulmonary disease both have elevated Kv11.1 channel expression in their lungs. Kv11.1 channels in lung tissues from both healthy humans and rats were limited to the big PAs.

Both the major and minor pulmonary arteries (PAs) of Kv11.1 channels were present in rats with pulmonary arterial hypertension (PAH) and in people with pulmonary hypertension linked to chronic obstructive pulmonary disease. The expression of Kv11.1 channels in PAH rats increased in tandem with the progression of changing pulmonary vascular structure throughout time. PAH-associated pulmonary vascular remodelling was reduced in PAH rats treated with dofetilide, a Kv11.1 channel blocker licensed by the FDA for the treatment of arrhythmias. Collectively, these findings highlight a new Kv11.1 channels' function in lung function and their potential as interesting targets for pharmacological intervention in managing hypertension. The fact that dofetilide has a protective effect suggests that this antiarrhythmic drug may one day be used to treat patients with pulmonary hypertension.

This study explicitly investigated Kv11.1 channel which is directly related to hERG gene showing its relation to other body systems such as the pulmonary system and investigating it as a possible pharmacological target.

Normal cardiac activity involves an excitation-contraction coupling that is well-balanced and coordinated, but it also involves intricate fluid exchanges between ionic currents in the membrane that are functionally related. On the other hand, their genetic studies offer crucial insights into how illnesses are controlled by their transcripts. Thus, in the tissues of the left ventricle resulting from dilated cardiomyopathy (DCM) or ischemia (ICM), Eda Seyma Kepenek and her colleagues at Ankara University, Ankara, Turkey analyse the most important voltage-gated ionic

channel gene expression levels, including sodium channels, Calcium channels, and potassium channels, both inward rectifier and delayed rectifier (Kepenek, E. S., et al 2020). Additionally, they looked at the mRNA levels of ERG-family channels (KCNH2) and ATP-dependent K+-channels. Additionally, the mRNA levels of CAMK2G, phospholamban, SR Ca2+-pump, protein kinase A, and ryanodine receptors were determined. SCN5A, CACNA1C, and CACNA1H mRNA levels declined significantly in the cardiac tissue samples of both groups with comparable importance, whereas KvLQT1 genes were elevated with decreasing Kv4.2.

While the KCNJ4 level was significantly high, the KCNJ11 and KCNJ12 in both groups were low. More significantly, only the ICM showed downregulation of the KCNA5 gene, whereas the DCM only showed upregulation of KCNJ2. All things considered, this work was the first to demonstrate that varying degrees of gene profile modifications caused by various cardiomyopathies are noticeable, especially in some K+-channels. This information adds to our understanding of how remodelling processes in HF can be distinguished between those that originate from various pathological conditions.

A coordinated effort by several ion channels produces both the AP essentially and the innate ion currents in the heart. The composition, absolute abundance, and proportionate ratio of ion channels define the electrical characteristics of heart cells. Researchers led by Balazs Ordog at University of Szeged, Szeged, Hungary used quantitative real-time PCR analysis to assess and contrast the expression levels of genes of a select group of cardiac ion channels among themselves and in relation to the human heart's ventricle and atrium (Ordög, B., et al 2006). The gene expression levels of 35 channels were examined, and it was found that there were significant differences in the gene expression levels of 21 channels between the ventricle and atrium of normal myocardium.

Furthermore, our investigation revealed that the presence of Kv1.7 and Kv3.4 mRNAs was notably high in both the atrium and ventricle. Additionally, the ventricle exhibited significantly elevated levels of Kv1.5 and Kv2.1 transcripts. These results might imply that these ion channel subunits serve a specific function in the stimulation of action potentials in the ventricle and atrium of the myocardium, respectively. This is the first study on the expression of Kv1.7, Kv3.3, and Kv3.4, among other ion channel subunits, in human heart cells. The levels of expression of these genes are similar to those of ion channel subunits that are well-known. Thus, it

is logical to assume that the native currents present in the myocardium may be mediated by these ion channel subunits.

HERG Gene

KCNH2 Function and Structure

The human ether a go-go-related gene type 1 (hERG1) K+ channel is encoded by the gene KCNH2, which was discovered in 1994. It was suggested that the rapid delayed rectifier K+ current, or IKr, is conducted by cardiomyocytes' hERG1 channel according to the biophysical properties of channels that were generated heterologously . IKr is one of numerous K+ currents involved in the repolarization of heart cells. It is distinct due to its pharmacological significance, link to a genetic kind of arrhythmia, and unusually quick inactivation rate in comparison to activation rate.

Long QT syndrome (LQTS)-related KCNH2 mutations may reduce IKr, which might culminate in sudden cardiac death and ventricular rhythm disturbances. As was previously indicated, hERG1 channels mainly assist in maintaining the resting potential in noncardiac cells.

Apart from the myocardium, many brain regions, tumour cells, gastrointestinal tract smooth muscle pancreatic β-cells, and the inner ear have all been found to harbour hERG1. Except for a rare correlation with epilepsy, no other organ is known to be impacted by KCNH2 mutations except the heart. Recently, a number of superb and thorough studies on hERG1 channels have been reported.

hERG1a and hERG1b Structural Differences

hERG1 is among the initial channels involved in the early stages of cardiac development, and its malfunction is linked to foetal death in the womb as well as sudden infant death syndrome. The KCNH2 gene produces various splice variations in hERG1 specific to heart tissues. Functional hERG1 channels consist of a minimum of two subunits encoded by different KCNH2 transcripts, namely hERG1a and hERG1b, which are nearly the same except for their N-terminal domains (Figure 7).

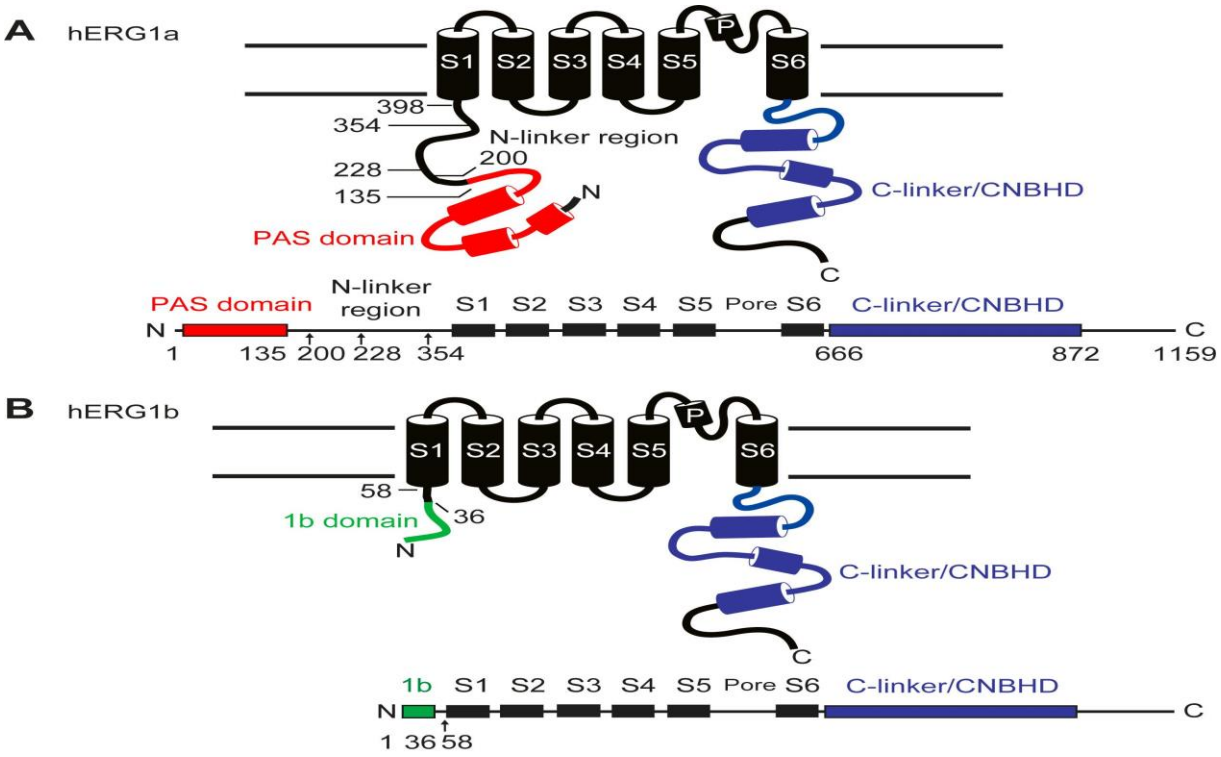

Figure 7: Scheme of hERG1a and hERG1b subunits (Johnson et al., 2022)

The N-terminal Per-Arnt-Sim (PAS) domain of hERG1a controls gating by dynamic interactions with the S4-S5 linker and the cyclic nucleotide binding homology domain (CNBHD) in the nearby proximal C-terminus. On the other hand, hERG1b lacks a functional PAS domain and exhibits a shorter and identifiable N terminus.

When contrasted to homomeric hERG1a channels, the recovery times for the activation, deactivation, and inactivation of heteromeric hERG1a/1b channels in HEK293 cells are twice as fast when the PAS domain is absent from hERG1b (Figure 8). The silencing of hERG1b in human cardiomyocytes through overexpression of a polypeptide mimicking the hERG1a PAS domain slumps the dynamics of gating of native Ikr and decreases its amplitude, resulting in an extended duration of action potential. Conversely, Ikr gating, Ikr magnitude, and cardiac repolarization are all accelerated when the hERG1a PAS domain is disabled with PAS-targeting antibodies. (McNally, B. A., et al 2017).

There is limited evidence supporting the genesis of heteromeric channels by hERG1a and hERG1b. When coexpressed in varying ratios, hERG1a and hERG1b RNA produced channels that have different deactivation dynamics from hERG1b channels, reaching a plateau. This suggests a proclivity of hERG1b subunits for

associating with hERG1a subunits. hERG1b demonstrates a higher tendency to interact with hERG1a rather than with other hERG1b subunits. This leads to the preferential formation of heteromeric ion channels consisting of hERG1a and hERG1b at the surface of the cell.

In the hearts of mammals, the primary alpha subunits responsible for generating the cardiac Ikr current, which aids in action potential repolarization, are the hERG1a and hERG1b isoforms. The hERG1a channels possess a lengthy N-terminal area that includes a regulatory EAG domain, on the other hand hERG1b lacks this domain. When expressed in a different biological system, hERG1a forms homotetrameric channels that exhibit substantial current flow and slow channel closure kinetics. Conversely, hERG1b forms homotetrameric channels with smaller current amplitudes and closing kinetics that are 5-10 times faster than those of hERG1a channels.

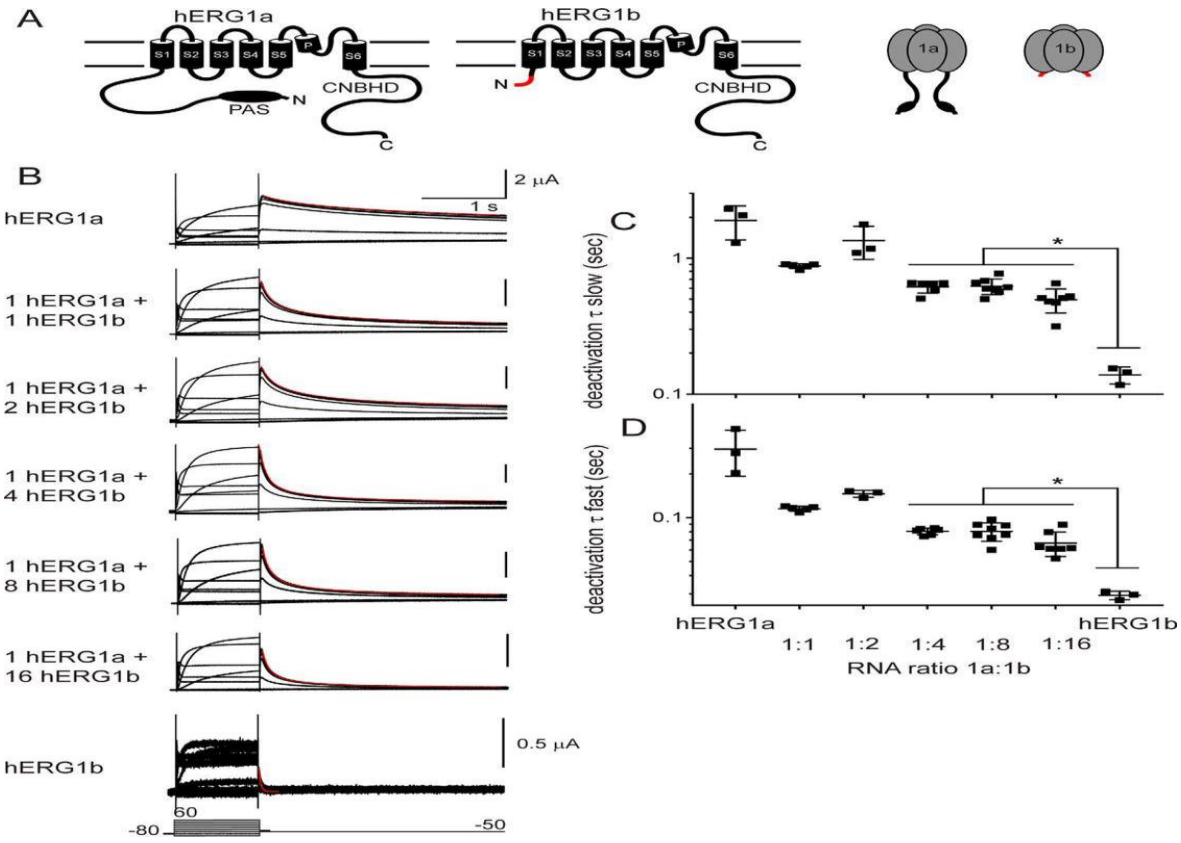

Figure 8: Coexpression of the human ERG1a and hERG1b subunits (McNally et al., 2017)

The rapid deactivation observed in hERG1b is attributed to the absence of the EAG domain, which is responsible for promoting slow deactivation in ERG1a. Both hERG1a and hERG1b are crucial for the formation of Ikr. Evidence from co-

immunoprecipitation studies in heart and brain cells supports the interaction between hERG1a and hERG1b subunits. Protein-interaction assays reveal that the The N-terminal domains of hERG1b and hERG1a channels interact with one another. Drugs exhibit varied inhibitory effects on currents in HEK293 cells expressing hERG1a alone compared to those expressing both hERG1a and hERG1b, indicating that LQTS may be significantly influenced by the makeup of the heart Ikr.

In homosapiens, the chromosome 7q35–36 contains KCNH2. The 16 exons that make up its coding region cover about 34 kb of DNA sequence. The hERG1 subunit, consisting of 1,159 amino acids, is the full-length variant (hERG1a). It is composed of six transmembrane domains (S1-S6) and has an estimated molecular mass of 127 kDa. The "eag domain," which is made up of residues 1 through 135 in the lengthy (376 amino acid) N-terminus of hERG1a, was discovered to be the first instance of a Per–Arnt–Sim (PAS) domain upon crystallisation. A PAS domain is a structure that facilitates interactions between proteins in a eukaryotic system.

Although the purpose of the hERG1a PAS domain is unclear, LQTS-related mutations in this area interfere with channel trafficking and quicken the rate of deactivation. This may be the consequence of interference with the PAS domain's ability to communicate with the channel's S4–S5 linker. The channel complex must be correctly folded in order for the PAS domain to be trafficked from the ER to the Golgi apparatus and cell surface. It is viable to phosphorylate the PAS domain.

Although hERG1b has a short N-terminus consisting of only 36 amino acids and does not possess the PAS domain, it is unable to reach the surface membrane independently. Instead, it requires coassembly with hERG1a subunits to facilitate its membrane localization. This is caused by its "RXR" ER retention signal sequence. Furthermore, it has been demonstrated that in mouse brains, erg1a and erg1b coassemble . The two hERG1a and hERG1b contain a cyclic nucleotide binding domain (CNBD) within their large C-terminus. cAMP has minimal effect on hERG1 channel gating, altering the voltage dependency of channel activation by a few millivolts, in contrast to cyclic-nucleotide gated channels, in which the opening of the channel requires cAMP adherence to the CNBD.

Active hERG1 channels are made up of four α-subunits, which are similar to other Kv channels and can be either hERG1a subunits only or hERG1a and hERG1b subunits. Two auxiliary β-subunits, MinK (KCNE1) and MiRP1 (KCNE2), can also coassemble with hERG1 proteins in heterologous expression systems. With a single

transmembrane domain and 123–129 amino acids, the KCNEs are tiny transmembrane proteins.

While MinK is most likely the accessory subunit that helps KCNQ1 produce IKs channels in the heart, when excessively expressed in systems of heterologous expression, it may as well change the density of the hERG1 channel. The gating dynamics and pharmacokinetics of hERG1were first demonstrated to be impacted by MiRP1, and mutations in MiRP1 associated with LQTS result in different alterations in hERG1 currents than does wild-type MiRP1 (Jain, A., et al 2023).

Studies utilising heterologous expression of MiRP1 have shown variation in its effects, and there has been disagreement about the implications and physiological importance of this exchange. Furthermore, it's unclear if physiologically meaningful levels of MiRP1 expression are restricted to the conduction system's Purkinje cells and pacemaker cells. leaving open the possibility that MiRP1 expression is elevated enough to affect the heart's overall hERG1 function. According to the most recent research, the link between hERG1 and MiRP1 may not be as physiologically significant as the relationship between KCNQ1 and MiRP1. Together with KCNQ1 and MinK, MiRP1 can create a heteromultimeric channel complex that reduces the total conductance of IK.

Furthermore, it has been shown that thyrocytes contain KCNQ1/MiRP1 channels that are inherently active without requiring external stimulation, and that adequate thyroid hormone production depends on these channels because of the targeted deletion of kcne2 in mice. LQTS linked to KCNE2 mutations may be exacerbated by impaired thyroid function. If a connection could be established between mutations in KCNE2 and the occurrence of arrhythmia or abrupt cardiac death in a sizable familial population, it would provide compelling evidence for MiRP1's involvement in Long QT Syndrome.

The Function of hERG1 in Repolarization of Action Potentials

Similar to other Kv channels, the activation of hERG1 is voltage dependent. About −50 mV is the voltage the channel opening threshold, while approx. −30 mV is needed for 1/2 activation. With a [K+] of 120 mM, the hERG1 channels' single channel conductance (γ) is 12.4 pS between −60 and −120 mV. At normal [K+] o (5 mM) values, nonstationary noise analysis estimates η to be around 2 pS at 0 mV. Moreover, the half-point of the voltage-dependent hERG1 channel inactivation is

around -85 mV. The slow rate of channel activation is in contrast to the quick onset of P-type inactivation, which is thought to be caused by the selectivity filter rearranging (Alameh, M., et al 2023).

The pace of channel deactivation during membrane repolarization is about ten times slower than the rate of channel reactivation after inactivation. The kinetic properties mentioned above have a significant influence on the extent to which hERG1 contributes to the overall current throughout the cardiac action potentials' delayed repolarization stage. The inactivation of the channel, which is more prominent at positive potentials across the membrane, substantially diminishes the immensity of Ikr during the cardiac activity potential's peak.

Channels recover from inactivation and move through phase 3 repolarization throughout the plateau stage. The slow process of deactivation causes the hERG1 current to momentarily increase in amplitude even while the electrochemical force for K+ outflow decreases.

Despite the fact that heteromultimeric hERG1a/1b channels are deactivated at a rate, that resembles the deactivation speed observed in actual cardiomyocytes, hERG1a channels deactivate at a slower pace compared to homotetrameric hERG1b channels. The gradual inactivation linked to hERG1a subunits was believed to originate from the interaction between the N-terminal PAS domain of the subunits and the S4-S5 linker. This linker serves as a structural connection that links the The motion of the electrical voltage sensor to the activation gate's opening and shutting. Deactivation is accelerated greatly by removing the PAS domain. It is possible to reverse delayed deactivation by coexpressing an additional N-terminal domain.

Other Cardiovascular Diseases correlated with hERG1 channels

Alterations resulting in a gain-of-function in heart potassium channels encoding genes, like KCNQ1 and KCNJ2, may provide a phenotype of QT interval that is incongruent with LQTS. Cardiomyocyte repolarization is accelerated by ECG. Short QT syndrome has been linked to a single missense mutation in hERG1 thus far. The voltage dependency of P-type inactivation shifts positively with the mutation Asn588. It is found within the S5-pore linker region of the hERG1 subunit. This lowers the degree of inactivation, and during the plateau stage of the action potential, a rise in outgoing current speeds up the ventricle's repolarization and shortens the QT interval. Mutations resulting in a loss of function in hERG1 or other K+ channels were discovered to induce fatal arrhythmias.; hence, drugs or gene therapies intended

to increase repolarizing currents in order to treat Long QT Syndrome (LQTS) must be meticulously developed to avoid excessive interventional activity.
It's interesting to note that, despite hERG1's widespread distribution throughout the nervous system, KCNH2 mutations have rarely been linked to epilepsy—rather than to any other neurological condition. Moreover, no reports of disease-causing hERG2 or hERG3 mutations exist. In CHO cells, the three erg protein types can unite to create heteromultimeric channels. Furthermore, voltage clamp experiments imply that heteromultimeric channels conduct currents, and using single-cell RT-PCR analysis, it was concluded that all three types of erg channels appear in the neurons of the rat embryonic rhombencephalon. There's a chance that malfunctioning hERG1, hERG2, and hERG3 channels will eventually be connected to new neuropathies.

While hERG1 channels are abundantly present in various organs, it appears that congenital mutations in KCNH2 primarily impact the heart in terms of disease manifestation. KCNH2 mutations are frequently the root cause of LQTS. The majority of mutations lead to subunit misfolding and/or decreased trafficking to the plasma membrane, hence compromising the channel's functionality. Even though our knowledge is limited about the involvement of hERG1 channels in epilepsy, muscle atrophy, and cancer, more research in this area is necessary.

hERG Sensitivity to Therapeutic Drugs

The hERG gene, encoding the Kv11.1 ion channels, plays a crucial part in regulating the potassium ion channels in the heart responsible for maintaining normal heart rhythm. However, its sensitivity to certain drugs poses a significant concern in pharmacology and drug development. Drugs that interact with hERG channels can cause QT interval prolongation, resulting in the development of dangerous cardiac arrhythmias, including Torsades de Pointes, which have the potential to be life-threatening. Thus, assessing the hERG gene's sensitivity to drugs is paramount in ensuring the safety and efficacy of pharmaceutical interventions, with rigorous testing protocols aimed at identifying and mitigating these risks during preclinical and clinical stages (Perry, M., Sanguinetti, M., & Mitcheson, J. 2010).

A study conducted in Canada by Shawn M. Lamothe et al demonstrated that the serine protease proteinase K (PK) selectively cleaved the hERG/IKr channel utilising whole-cell patch clamp and Western blotting analysis (Lamothe, S. M., et al 2016). Using molecular biology techniques, researchers have identified the S5-pore

linker of the hERG protein as the specific domain targeted by proteinase K cleavage. This determination was made by creating a hybrid channel between the protease-sensitive hERG and the protease-insensitive human ether-a-go-go (hEAG), and then applying the spider toxin BeKm-1. In a rabbit model, they established a connection between myocardial ischemia and a decrease in expression of mature ERG. Additionally, we observed an elevation in many proteases, such as calpain. This investigation aimed to examine the physiological significance of the heightened vulnerability of hERG proteases in the context of myocardial ischemia. Applying methods from molecular biology we discovered that calpain-1 cleaved hERG at the S5-porelinker after being Introduced into the surrounding extracellular environment through an active release process. By using site-directed mutagenesis and protease cleavage-prediction tools, we were able to determine that calpain-1 enzyme specifically split hERG at the Gly-603 position within the S5-pore linker region of the hERG protein. Definement of protease-induced hERG disruption broadens our comprehension of hERG regulation. Proteases like calpain can cause damage to the hERG, which can lead to QT prolongation associated with ischemia and sudden cardiac death.

Other Mutations that can affect KCNH2 gene

Sudden Cardiac Death (SCD) exhibits daytime fluctuations. Research indicates that the timing of sudden cardiac death (SCD) is influenced by circadian variations in the modulation of the electrical properties of the heart, both at the neurohumoral level and specifically within the cardiomyocytes.Certain genes display circadian expression patterns that are regulated by two transcription factors called CLOCK and BMAL1. These transcription factors belong to the basic helix-loop-helix family. Elizabeth A. Schroder et al in the University of Kentucky, Lexington, investigated the possibility that potassium channel expression and diurnal variations in ventricular repolarization are influenced by Bmal1 expression in cardiomyocytes (Schroder, E., et al, 2015). They used transgenic mice that induced the deletion of Bmal1 that precisely affects cardiomyocytes (iCSΔBmal1-/-). We employed voltage-clamping, electrocardiographic (ECG) telemetry, promoter reporter bioluminescence experiments, and quantitative PCR. While iCSΔBmal1-/- mouse hearts showed downregulation of multiple K+ channel gene transcripts, only KCNH2 presented a

distinct circadian expression motif, which was disturbed in the myocardium of iCSΔBmal1-/- mice. In ventricular cardiomyocytes lacking the iCSΔBmal1-/- mutation, the Kcnh2 gene responsible for driving the rapidly activating delayed-rectifier K+ current showed a significant reduction. Specifically, the observed IKr in these mutant myocytes was approximately 50% lower compared to normal myocytes. Experiments using promoter-reporter tests illustrated that the Kcnh2 promoter is activated by the co-expression of BMAL1 and CLOCK transcription factors. During the light (resting) phase, ECG analysis revealed that iCSΔBmal1 /- animals had a lengthening in heart rate QTc interval. The uncorrected QT interval showed an enhanced circadian rhythm, while the RR interval did not alter in tandem. The circadian expression of Kcnh2 is regulated by the heart's internal clock., along with other K+ channel genes. This molecular clock is essential for ensuring correct ventricular repolarization. The interruption of the circadian clock system in cardiomyocytes is anticipated to reveal variations in ventricular repolarization throughout the day, which may be linked to a higher risk of SCD and cardiac arrhythmias.

Another research done by Sara Ballouz et al at the University of New South Wales using utilising extensive gene expression datasets to carry out a co-expression study meta-analytically, with the goal of identifying important cardiac ion channel gene modules that are co-expressed in human hearts (Ballouz, S.et al, 2021). A robust association between the CACNA1C expression and KCNH2 expression was found through the analysis of 3000 publicly available human RNA sequence datasets. This link was noted as well in cardiac tissue sampled for adults. It was found that the co-expression of KCNH2 and CACNA1C would decrease vulnerability to early depolarizations and restrict the alterations in AP durations observed with differences in ion channel gene expression through in silico modelling. Additionally, they discovered that the expression of KCNH2 and CACNA1C are connected in hiPSC-CMs, and that the extent to which the length of repolarization alters after IKr inhibition is negatively correlated with the expression of CACNA1C and KCNH2. Gene modules that are crucial for controlling cardiac physiology can be found using meta-analytic techniques applied to several independent human gene expression datasets. In particular, we have confirmed that the ion channel genes CACNA1C and KCNH2 are co-expressed in cardiac tissue.

Additionally, in silico investigations indicate that when co-expressed together, CACNA1C and KCNH2 enhance the heart's electrical activity's resilience.

Genetic variables have a role in the substantial phenotypic diversity observed in relatives who share the same mutation having LQTS. A study was conducted by Ricardo Caballero and his colleagues at Universidad Complutense, Madrid, Spain to find the underlying cause (Caballero, R., et al, 2017).. Two related individuals diagnosed with LQTS in a family from Spain of African descent had two mutations. One in the TBX20 gene which encodes for the transcription factor Tbx20 and the other in the KCNH2 that is the coding gene for Kv11.1 ion channels discovered by next-generation sequencing. We functionally examined these variants. A heterozygous mutation in KCNH2 that codes for p.T152HfsX180 Kv11.1 (hERG) is present in affected relatives. After transfecting these mutations into CHO cells, this peptide by itself was unable to produce any current; yet, remarkably, it had "chaperone-like" impacts on native hERG channels in these cells.

As a result, the current produced in these cells that comprise the transfection of native (WT) and Kv11.1 channels was identical to the current produced by the WT channels alone. hiPSC-CMs showed an increase in the expression of human KCNH2 gene, shorter action-potential duration (APD), and increased hERG currents (IhERG). Tbx20, however, exhibited no impact on the expression or function of other repolarization related channels. On the other hand, hiPSC-CMs with the Tbx20 mutation had no increased KCNH2 expression, which resulted in an increase in APD and a decrease in hERG current.

According to the findings, Tbx20 regulates the expression of hERG channels, which are in charge of producing the delayed rectifier current's fast component. Conversely, Tbx20 p.R311C selectively inhibits Tbx20 pro transcriptional activity over KCNH2. Thus, it is possible to classify TBX20 as a KCNH2-modifying gene.

Gaps in Research and Studies

After researching the topic of cardiovascular diseases in general, and coronary artery disease in particular, it is obvious that this topic is vastly under studied. This may be due to the complex nature of this disease, as it is not caused by a single mutation or a certain gene. It is a compilation of genetic as well as environmental factors.

Many genes have been studied and found to have a correlation or a causation relation with CAD including but not limited to genes responsible for nitric oxide supply, cholesterol regulation and ion balance in the body. The hERG gene is the gene encoding for the rapid repolarization of K channels. A mutation in this particular gene has shown to have a huge impact on the cardiovascular system. This gene was correlated with various heart condition such as ischemic heart disease, long QT Syndrome as well as arrhythmias and heart failures.

However, despite the large number of studies investigating hERG, very little has been said about its relation with CAD.

Ion channels are the gate to the heart, hence well regulated ion channels provide a good heart. On the other hand any imbalance whether downregulation or upregulation in any ion channel will lead to cardiovascular disease. hERG coding for the rapid repolarization of the K+ channels it is vital to study its mutations, pathways and levels of expression.

This study will try to shed some more light on the expression levels of hERG in CAD patients, to better understand its role in this disease.

Conclusion

To conclude this literature review, the key findings of the past studies proves the complexity of coronary artery disease with few known related genes. CAD also is affected by environmental factors as well as other cardiovascular diseases. Past research focused on the association ofLong QT Syndrome with the severity of CAD. The majority of these studies agreed on the fact that as coronary artery disease (CAD) becomes more severe, the duration of the QT-interval decreases. leading to arrhythmias. The hERG gene is accountable for LQTS. This gene coding for the subunit playing a part for forming the pore of the rapidly activating delayed rectifier potassium channel , which serves a crucial part in the repolarization of the heart.This gene has also been correlated with other cardiovascular diseases and have a significant role in the voltage gated ion channels which fuel the whole cardiovascular system. Studies also proved the sensitivity of hERG to many therapeutic drugs as well as other mutations. However, few studies have linked hERG to CAD although evidence points to a clear connection. Hopefully this study will contribute more to the knowledge and shed light on this connection.

CHAPTER III

Methodology

Materials

Chemical Reagents and Kits

Kits for RNA isolation, cDNA synthesis, and 2X SYBR Green qPCR Mix were all obtained from Hibrigen, Turkey and used in the investigation.

Equipment

Sanyo MDF-U537 Biomedical Freezer (Japan), Eppendorf 5417R Refrigerated Microcentrifuge (Massachusetts, USA), Sigma 3-18KS Centrifuge (Osterode am Harz, Germany), MetiSafe® Laminar Air Flow Cabinet (Ankara, Turkey), MetiSafe® PCR Cabinet (Ankara, Turkey), Nano-drop™ 2000/2000c Spectrophotometer (Thermo-scientific™, Pittsburgh, USA), SimpliAmp™ Thermal Cycler (Applied Biosystems™ by Thermo Fisher Scientific™), RotorGene Real-Time PCR (Qiagen, Hilden, Germany), Spin (Qiagen, Hilden, Germany). Thermo Scientific™ Herasafe™ KS, Class II Biological Safety Cabinet, PowerPRO 300 Power Supply, 300V, 700mA, 150W (Cleaver Scientific™, Rugby, UK), and DNR Bio Imaging Systems MiniBiS Pro (DNR Bio Imaging Systems™, Neve Yamın, Israel).

Oligonucleotides

The primers that were used in this study were procured from Olgomer, Turkey.

Other Chemical Agents

Agarose powder (Sigma-Aldrich, catalogue number 11388983001) was employed for gel electrophoresis to visualise PCR product samples. Ethidium bromide (EtBr) (Sigma-Aldrich, catalogue number E1385) and TAE Buffer.

Computer Software

The Gel Capture Software version 4.25 facilitated the viewing, analysis, and storage of gel images and imaging data. DNA quantification was performed using the Nano-drop™ 2000/2000c software. Data statistics were conducted using the Statistical Package for the Social Sciences (SPSS).

Ethical Clearance

The Ethical Committee for Scientific Research at Near East University approved the collection of samples (1748 2024/06/ 21).

Methods

Research Design

Retrospective cohort methodology was employed in this research that allowed for examining the connection between being exposed to specific risk factors and the incidence of CAD by looking back at data that has already been gathered. According to the study design, a cohort of 32 individuals who have received a diagnosis of coronary artery disease was identified, and a control group was made up of 36 people who have not been diagnosed with the aforementioned condition. The purpose of this research is to see if there is any connection between hERG1a, hERG1b expression, and how CAD develops by comparing the two groups. The study was carried out in compliance with the moral guidelines. governing medical investigations utilising human samples as well as the guidelines outlined in the World Medical Association Declaration of Helsinki. Additionally, the Near East University Hospital (NEUH) and the ethical review board approved it.

Participants/Population and Samples

The subjects of this study are patients who are being treated at the NEUH. In order to identify people who had been diagnosed with the disease of interest, coronary artery disease, the study gathered data from the hospital system. 32 individuals who had been diagnosed with coronary artery disease comprised the patients' group, ECGs (QT assessments) were utilised to confirm

the diagnosis and look for LQTS. The control group consisted of 36 healthy individuals.

Data Collection Tools

The hospital information system yielded the patient records, which covered the years 2022–2024. The search was conducted using a few keywords, such as "heart attack," "coronary artery disease," "long QT syndrome," "ischemic heart disease," and "heart failure." Individuals who met the criteria for a CAD diagnosis were selected, and the negative control group was made up of patients with no arterial anomalies or plaques.

Gey's Buffer Preparation

Gey's solution, also known as Gey's balanced salt solution (GBSS), is a type of nutrient medium or solution used in cell culture and tissue preservation. GBSS is a balanced salt solution that is designed to provide the necessary minerals, ions, and nutrients to support the growth and maintenance of cells in laboratory settings. It typically contains a mixture of various salts, including calcium, magnesium, potassium, and sodium chlorides, in specific concentrations. The solution is usually supplemented with glucose or other energy sources to support cell metabolism. To prepare the solution, the specified quantities of the ingredients were combined and thoroughly mixed before undergoing autoclaving. The following are the amounts of the individual components utilised.

Table 1. The components of Gey's Buffer

Components	Volume
NH_4Cl	8,291 grams
$KHCO_3$	1,001 grams
dH_2O	1 litre

Leukocyte Isolation

Leukocyte isolation from whole blood commenced with the initial suspension of whole blood obtained from EDTA tubes in GBSS to lysate red

blood cells. Subsequently, the samples underwent a 30-minute incubation period in a refrigerator, followed by centrifugation using the Sigma 3-18KS Centrifuge (Osterode am Harz, Germany). After discarding the supernatant, the remaining blood was resuspended in GBSS and subjected to another round of centrifugation, with subsequent removal of the supernatant. Finally, the resulting pellet was resuspended in Phosphate-buffered saline (PBS) solution and aliquoted into 4 Eppendorf tubes, each with a volume of 250uL, before storage at -20^{o}C.

RNA Extraction

The leukocytes that were previously isolated provided the RNA. After this, the leukocytes were isolated and homogenised in a solution containing 0.5 millilitre of TRIZOL reagent. Subsequently, 0.25 millilitres of chloroform were added to every 0.5 millilitre of TRIZOL reagent, and the tubes were sealed. The samples were vortexed for 10 seconds, then they were put at a temperature of 25-30°C for 2-3 minutes. Centrifugation was performed on the samples after the incubation period. The process was carried out at a force of 12,000 ×g for 15 minutes, at a temperature of 2 to 8°C. Centrifugation was employed to separate the mixture into several phases, which included an upper phase made up of a colourless aqueous solution, an intermediate phase, and a lower phase that was reddish-coloured and comprised of phenol-chloroform. The RNA was carefully transferred to fresh tubes, being cautious not to disturb the interphase. RNA was combined with propanol alcohol in order to cause the RNA to separate from the aqueous phase. Then, for every millilitre of the previously used TRIZOL reagent, 0.5 millilitres of that combination were added. Subsequently, the mixture was incubated at fifteen to thirty degrees Celsius for ten minutes. A gel-like particle made of RNA was seen after centrifugation at a maximum force of 12,000 x g for 5 minutes at 2 - 4°C. This pellet accumulated along the centrifuge tube's bottom and side; it was not visible before centrifugation. After the supernatant was completely removed, A minimum of 0.5 millilitres of 75% ethanol was used to wash the RNA pellet. for every 0,5 ml of the TRIZOL reagent that was previously used. Two times between 2 and 8°C, the vortexing and centrifugation processes were carried out at a maximum speed of 7,500 x g for five minutes. In order to guarantee the total removal of any leftover ethanol,

the RNA pellet was left to dry in the open for a maximum of 10 minutes; vacuum centrifugation was purposefully avoided in order to minimise over drying. 50 µl of nucleases free water was added to the pellet in order to elute the RNA.

RNA Quantification

A nanodrop spectrometer (Thermo-Scientific, Pittsburgh, USA) was selected as the technique for determining the concentration and purity of RNA because of its many benefits, which include its ease of use, low sample volume need, affordability, and widespread commercial availability. The Nanodrop instrument is a spectrophotometer intended for microvolume analysis that uses UV-Vis spectroscopy methods to assess protein and nucleic acid (NA) sample concentration and purity. With this method, NA concentrations as low as 1 ng/l can be properly measured. While measures of concentration and purity are generally accepted as trustworthy, a deeper analysis of the data can provide important information about the sample's quality and suitability for other uses. The amount of RNA can be measured using ultraviolet (UV) spectroscopy by measuring the absorbance of an RNA sample at specific wavelengths, such as 260 and 280 nm. In order to calculate the concentration of nucleic acids, the Beer-Lambert equation is utilised, which states that absorbance and concentration have a proportional relationship. The A260/A280 ratio provides useful information on the overall evaluation of its purity; a ratio of 1.8 to 2.0 is deemed optimal. In this ratio, a greater value often indicates the presence of protein contamination, whereas a lower value indicates the presence of RNA contamination with other chemicals like salt and phenol. It was found that the RNA concentration and purity were ideal and suitable for the objectives of the investigation.

Complementary DNA (cDNA) Synthesis

Hibrigen cDNA synthesis kits, which include a number of necessary components, were used to carry out the cDNA synthesis. Reverse transcriptase and RNase inhibitors were combined to form the enzyme mix solution, which was a necessary ingredient. A well-calibrated buffer environment held dNTPs, MgCl2, random 6-mer primers, and oligo-dTs in the reaction solution, which

was painstakingly prepared for optimal efficiency. Among these components, the attached oligo (dT) primer was crucial to the field of molecular biology. This 18-nucleotide primer's function is to bind to the 5' end of the poly(A) tail, ensuring efficient complementary DNA (cDNA) synthesis. In molecular biology, the Random Hexamer Primer is a commonly utilised primer for randomly priming RNA molecules. Nuclear-free water is the final component utilized in the synthesis process, which increases the overall precision of the reaction. As Table 6 illustrates, the entire cDNA synthesis procedure took one hour and ten minutes.

Table 2. Describes the Protocol for cDNA Synthesis

Component	**1x**
Enzyme Mix	1 µL
Reaction Buffer	4 µL
dNTPs	1 µL
Primers (Oligo dt)	1 µL
Nuclease-Free dH2O	3 µL
Total RNA	10 µL

Table 3. demonstrates the conditions for polymerase chain reaction in cDNA synthesis.

Step	**Temperature**	**Time**
cDNA Synthesis	50°C	15 Minutes
Inactivation of Kit	85°C	5 Minutes

Primer Design

Primers for hERG1a and hERG1b were designed utilising Primer Blast. Qualities such as product length, self complementarity and GC content were carefully considered before choosing the best primer pairs.

Table 4. identifies the target base sequences for the primer pairs 5'-3’

hERG1a	Product Length 143	**Forward**	CAC TGA CTG CCA TGT GAC CT
		Reverse	GAG CGT TGG CGA TGA TGA AAC

hERG1b	Product Length 149	**Forward**	CAG CAA CAA GCT GGT AGA
		Reverse	GAT GGA TCC TGG AAC CCA AG

Primer Optimization using gradient PCR

In this work, a temperature gradient ranging from 56°C to 62°C was used to evaluate multiple annealing temperatures in order to determine the most optimal temperature for the primers. This process facilitates the determination of the optimal temperature for the target sequence's amplification to be most successful. This ensures that, within the constraints of the experiment, the annealing temperature is selected to maximise the PCR's efficacy. Gradient PCR was performed using the SimpliAmpTM Thermal Cycler (Applied Biosystems by Thermo Fisher Scientific, catalogue number: A24811) to determine the optimal temperature settings for real-time PCR.

Table 5. Optimal parameters used for gradient PCR

	Stage	Temperature	Time	Cycles
Steps	Initial denaturation	95℃	5 mins	
	Denaturation	95℃	30 sec	35 cycles
	Annealing	56℃ - 62℃	30 sec	
	Extension	72℃	40 sec	
	Final extension	72℃	7 mins	

Table 6. displays each gene's final amount of both forward and reverse primer.

Primer	**Volume**
hERG1a	0.50 µM
hERG1b	0.50 µM

Table 7. Specifies PCR Conditions: Annealing

Primer	Temperature	Time
hERG1a	54°C	30 Seconds
hERG1b	58°C	30 Seconds

PCR Product Analysis

The amplified products were evaluated using a PCR product analysis. The PCR results were analysed through the application of gel electrophoresis. The process comprises using an agarose gel to separate the amplified DNA fragments according to size. By comparing the fragmented samples with a molecular weight marker, the expected size of the amplified product is verified. Concurrently, this investigation confirms the specificity of the amplification process by enabling us to locate and verify the absence of non-specific bands or artifacts. It was possible to ascertain the accuracy of the PCR amplification process by conducting a comprehensive examination. This entails confirming that the final products match the anticipated size and are free of undesired or non-specific amplification products. This critical stage is included to check that these optimised primer conditions are reliable and accurate, indicating that they are suitable for use in later experiments.

Quantitative PCR (qPCR) for B-actin housekeeping gene

The fundamental cellular processes necessary for every cell's survival and upkeep depend on housekeeping genes. These genes are frequently employed as reference or control genes in PCR to standardise the expression of the target genes that are being amplified. Housekeeping genes, including 18S rRNA, β-actin, and GAPDH, are often expressed in a consistent manner in a variety of cell types, tissues, and experimental setups. Researchers can adjust for variances in the initial concentration of RNA or DNA, as well as variations in the effectiveness of the reverse transcription and PCR amplification stages, by employing a housekeeping gene as an internal reference. This makes it more likely that any changes in the target gene's expression that are seen are caused by real biological differences rather than by experimental or technological errors. For PCR-based methods to quantify gene expression accurately and reliably, appropriate housekeeping gene selection and validation are essential.

Because it consistently expresses itself in a variety of cell types and experimental settings, the β-actin gene is frequently utilised as a housekeeping gene in PCR-based investigations. It acts as a crucial internal control, enabling scientists to adjust for variations in the starting material, the efficiency of reverse transcription, and other technical aspects that may affect the outcome of PCRs, as well as to normalise the expression of their target genes.

For the real time PCR SYBR green, primers, ddH2O and cDNA were added to the PCR tubes. After which the machine was set to run, and results were uploaded to the connected computer presented as a separate curve for each sample.

Table 8: demonstrate the protocol used for qPCR

Component	**1x**
2x SYBR Green Probe	5 µL
ddH2O	1 µL
B-actin Forward Primer	0.5 µL
B-actin Reverse Primer	0.5 µL
cDNA	3 µL

Real Time Polymerase Chain Reaction (Quantitative Polymerase Chain Reaction qPCR)

This approach was selected because of its exceptional accuracy, reactivity, and specificity in determining the levels of gene expression. In addition to the fundamental ingredients, primers like Taq DNA polymerases, dNTPs, potassium chloride (KCl), magnesium chloride (MgCl2), template cDNA, and SYBR Green are used in the amplification of cDNA. A 2x SYBR Green probe is used for polymerase chain reaction (PCR) real-time monitoring. The fluorescence signal shows a positive correlation with the quantity of amplified DNA as the PCR proceeds. By comparing fluorescence signals with reference genes, quantitative methods for determining gene expression levels can be used to determine the initial amount of RNA present in the sample.

Table 9. Demonstrates the Protocol of the qPCR Experiment: 35x Cycles

Component	hERG1a (1x)	hERG1b (1x)
2x SYBR Green Probe	5 µL	5 µL
Forward Primer	0.5 µL	0.5 µL
Reverse Primer	0.5 µL	0.5 µL
dH2O	1 µL	1 µL
cDNA	3 µL	3 µL

Data Analysis Procedures

SPSS software (Statistical Package for the Social Sciences 25.0, SPSS Inc., Chicago, IL, USA) was used to do the statistical analysis. The information was displayed as mean ± standard error (SE). Cycle Threshold (Ct) values, which denote the cycle number at which logarithmic PC plots intersect a threshold line that has been calculated, were obtained as the gene expression data. The expression of each gene was compared between depots using the 2ΔΔCt technique. By deducting the target gene's Ct value from the housekeeping gene's Ct value, the ΔΔCt value is determined using this method. The Student's t-test and the Mann-Whitney U test were used in the study to compare continuously distributed continuous variables that were normally distributed and those that were abnormally distributed. The relationship between the QTC values of CAD patients and their gene expression levels was investigated using Spearman correlation analysis. Less than 0.05 was the threshold for statistical significance.

CHAPTER IV

Findings and Discussion

Title: Findings for Research Question I

After running the qPCR for B-actin, hERG1a and hERG1b the following results were obtained. The results will be separated into three main parts. The first one will compare the findings regarding the QT assessment between patients and control group. The second part will present the results comparing the hERG1a and hERG1b mRNA expression between patients and control group. And the third part will combine all this data together.

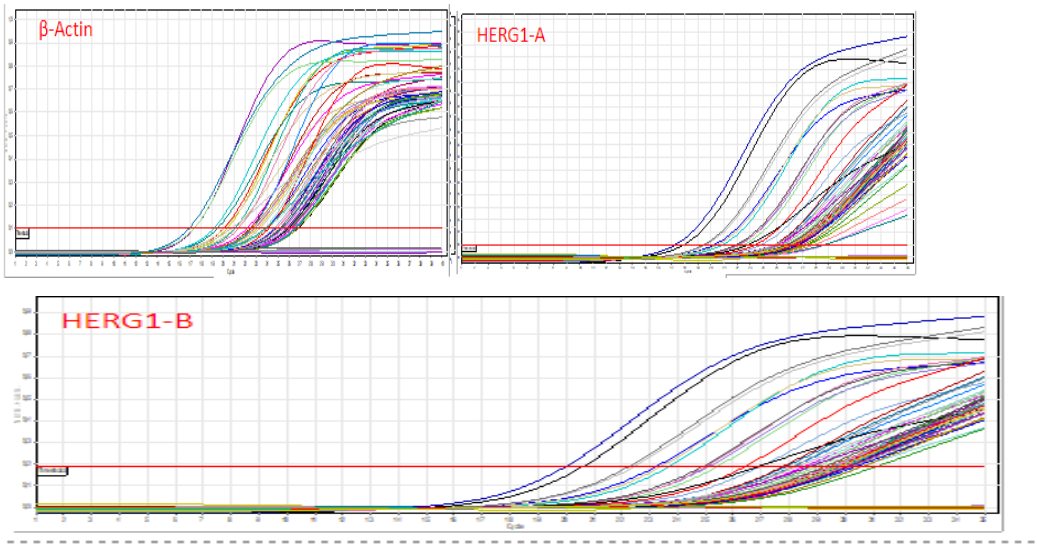

Figure 9: Graphic shows of the qPCR product of B-actin, hERG1a and hERG1b respectively

Comparison between the QT assessments of the patients' group and the control group

QT Interval

The mean QT interval for the patient group is 410.72 ms, with a standard deviation of 25.93 ms and a standard error of 4.81 ms. The mean QT interval for the control group is 383.09 ms, with a standard deviation of 7.24 ms and a standard error of 1.54 ms. The difference in mean QT interval between the patient and control groups is 27.63 ms, which is a substantial difference. The larger standard deviation in

the patient group suggests more variability in QT intervals compared to the control group.

QTc Interval

The mean QTc interval for the patient group is 421.17 ms, with a standard deviation of 12.86 ms and a standard error of 2.39 ms. The mean QTc interval for the control group is 390.27 ms, with a standard deviation of 14.41 ms and a standard error of 3.07 ms. The difference in mean QTc interval between the patient and control groups is 30.90 ms, which is a large difference. The variability in QTc intervals appears to be similar between the two groups.

Heart Rate

The mean heart rate for the patient group is 66.17 bpm, with a standard deviation of 11.21 bpm and a standard error of 2.08 bpm. The mean heart rate for the control group is 68.45 bpm, with a standard deviation of 16.00 bpm and a standard error of 3.41 bpm. The difference in mean heart rate between the patient and control groups is 2.28 bpm, which is a relatively small difference. The variability in heart rate appears to be higher in the control group compared to the patient group.

Key findings

The patient group has significantly prolonged QT and QTc intervals compared to the control group. The variability in QT intervals is higher in the patient group. The difference in heart rate between the groups is relatively small. These results suggest a strong correlation between coronary artery disease and the prolonging of the QT/QTc intervals.

	Samples	N	Mean ± SD
QT	Patients	29	410.7241, 25.92695
	Control	22	383.0909, 7.24345
QTc	Patients	29	421.1724, 12.86487
	Control	22	390.2727, 14.41290
Heart rate	Patients	29	66.1724, 11.20609
	Control	22	68.4545, 15.99026

Table 10. Table comparing the group statistics of the QT, QTc and heartrate between patients and control groups.

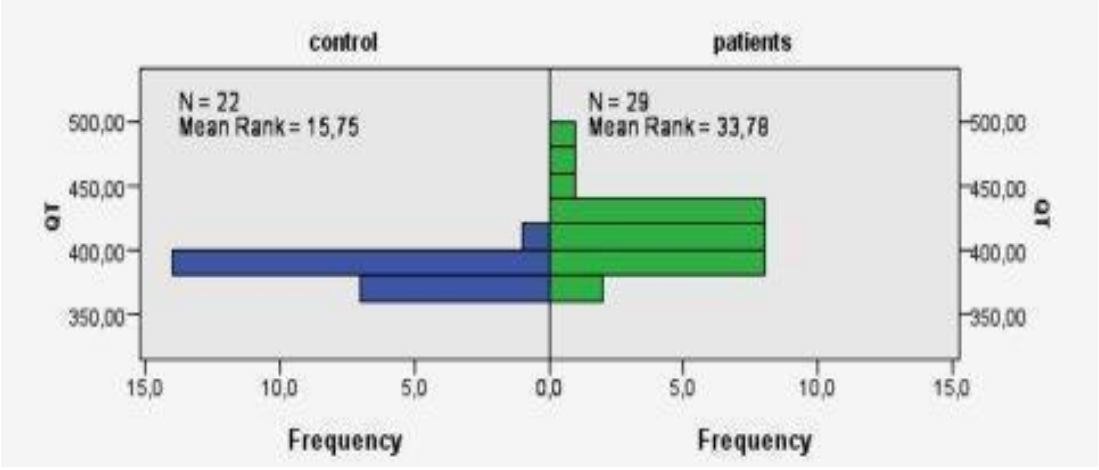

Figure 10: Bar graph comparing the frequency of QT interval between patients and control group.

This bar graph indicates that the control group consisting of 22 individuals has a mean value of around 10.75, while the patient group has a higher mean value of around 33.18 with 29 individuals. The difference between the two groups is statistically significant with p=0.001, indicating that patients showed significantly longer QT segments. This led to the deduction of a positive correlation between coronary artery disease and long QT syndrome.

The QT interval represents the time from the start of the Q wave to the end of the T wave on an ECG. It reflects the total duration of ventricular depolarization and repolarization. The QT interval varies with heart rate - it is shorter at faster heart rates and longer at slower heart rates.

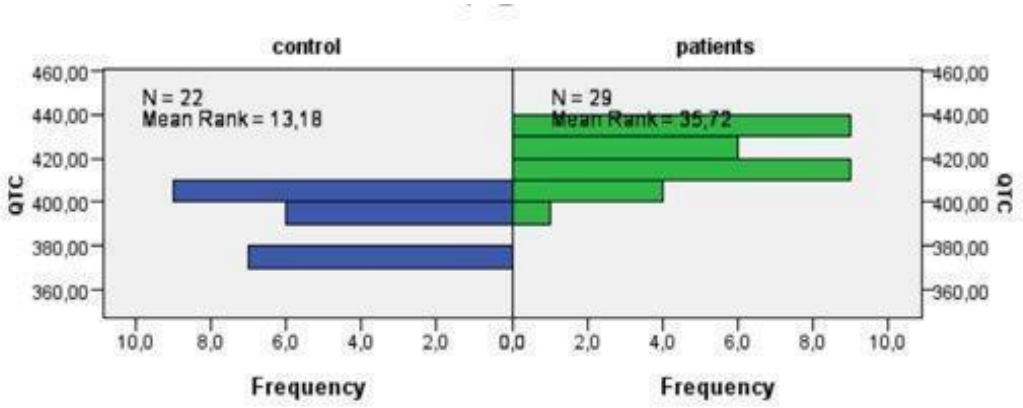

Figure 11: Bar graph contrasting QTc between patient group and control group

In this barograph comparing the QTc of the patients and the control group, the control group has a mean rank around 13.18, while the patient group has a higher mean rank around 35.72. The distributions appear skewed, with most control values clustered around the lower frequencies and most patient values clustered around higher frequencies, though there is some overlap. The difference between the two groups is statistically significant, with a p=0.001.

The QTc interval is the QT interval corrected for the heart rate. It is calculated using a mathematical formula, such as the Bazzett formula: QTc = QT / √RR. The correction accounts for the inverse relationship between QT and heart rate. QTc provides a more standardized measure of ventricular repolarization that is less dependent on the heart rate. QTc allows for comparisons of ventricular repolarization duration between individuals or conditions with different heart rates. QTc is a better predictor of arrhythmia risk and sudden cardiac death compared to QT interval alone. Many clinical conditions and medications can prolong the QT interval - using QTc helps distinguish pathological QT prolongation from normal variations due to heart rate. In summary, QTc is a heart rate-corrected measure of ventricular repolarization duration that provides a more standardized and clinically relevant assessment compared to the raw QT interval. Both metrics are important in cardiovascular evaluation and risk assessment.

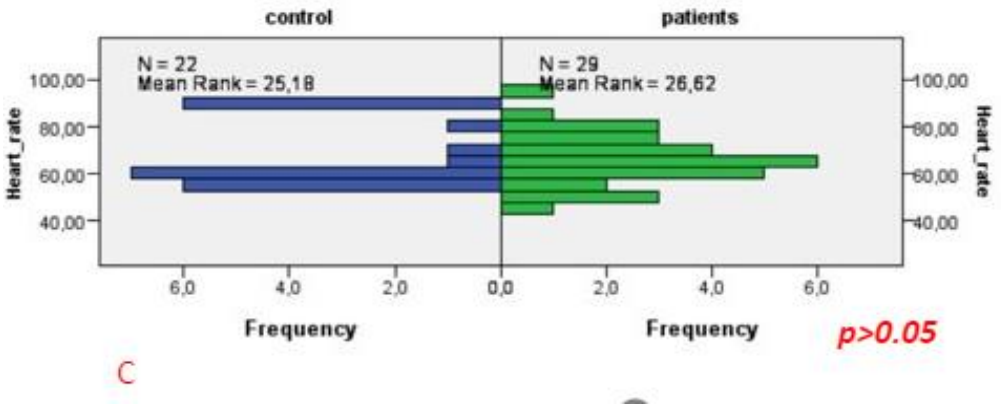

Figure 12: Barograph comparing the heart rate of the patient and the control group.

The control group has a mean rank around 25.18, while the patient group has a slightly higher mean rank around 26.62. The distributions appear more symmetric and overlapping compared to the previous graphs, suggesting less separation between the control and patient groups in the heart rate. No statistically significant difference was found between the groups (p=0.552), indicating no correlation between heart rate and CAD.

Comparison of hERG1a and hERG1b mRNA expression between patients and control group

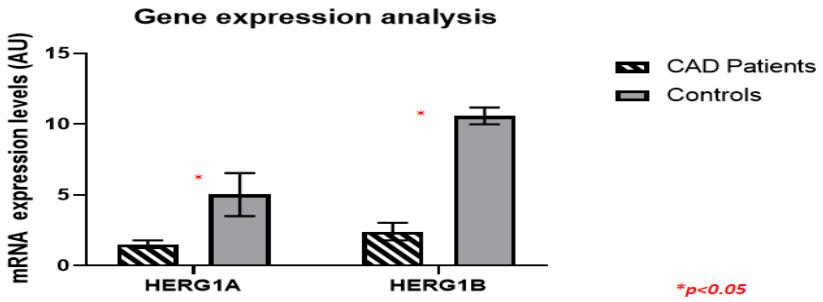

Figure 13: Bar graph comparing the mRNA expression levels of hERG1a and hERG1b between CAD patients and control group

The bars in this figure represent the mean RNA expression levels, with error bars indicating the variation within each group. For HERG 1A, the expression is slightly higher in controls compared to CAD patients, while for HERG1B, the expression is significantly higher in CAD patients,(p=0.001, p=0.017 respectively).

This graph indicates that hERG1a and hERG1b mRNA expression are positively correlated meaning that a decrease or increase in the expression of the first gene is followed by a decrease or increase in the expression of the second gene respectively.

The gene expression data was acquired in the form of Cycle Threshold (Ct) values, where Ct represents the cycle number at which logarithmic PC plots intersect a calculated threshold line. In this research, gene expression data was obtained through quantitative polymerase chain reaction (qPCR). The data is typically represented in the form of Cycle Threshold (Ct) values.

During qPCR, the genetic material (DNA or RNA) is amplified through a series of cycles. The Ct value represents the cycle number at which the amplification reaches a certain threshold. This threshold is determined by analysing the logarithmic plots of the PCR amplification curves. To determine the Ct value, the PCR machine measures the amount of fluorescence emitted during each cycle. As the amplification progresses, the fluorescence increases. The Ct value corresponds to the cycle number at which the fluorescence signal crosses the threshold line.

The Ct value is inversely proportional to the amount of target genetic material present in the sample. A lower Ct value indicates a higher abundance of the target gene, while a higher Ct value suggests a lower abundance. Ct values were utilised to compare gene expression levels between different samples or conditions. It helps in understanding which genes are more or less active in specific biological processes or experimental conditions.

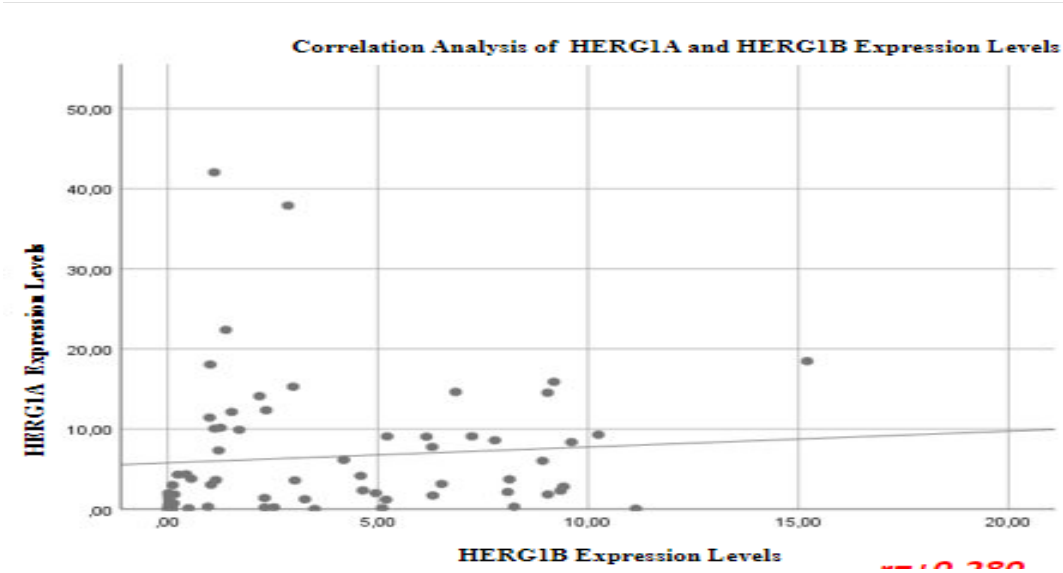

Figure 14: Line graph illustrating the correlation analysis of hERG1and hERG1b

The x-axis on this line graph represents the expression levels of HERG 1A, while the y-axis represents the expression levels of HERG 1A. Each data point on the scatter plot represents an individual observation or sample, plotting the expression level of HERG 1A against the expression level of HERG1B. There appears to be a positive correlation between the two variables, as indicated by the upward-sloping trend line fitted through the data points. The correlation coefficient (r) is calculated as 0.280, suggesting a moderate positive correlation between the expression levels of HERG 1A and HERG1B. Additionally, the p-value is found 0.024, indicating that the observed correlation is statistically significant at the 5% significance level. Overall, the results suggest that higher expression levels of HERG1B are associated with higher expression levels of HERG 1A, and this relationship is moderately strong and statistically significant.

The 2ΔΔCt method was employed to compare the expression of each gene between depots. In this method, the ΔΔCt value is calculated by subtracting the Ct value of the target gene from the Ct value of the housekeeping gene. Once we have the ΔCt values, we proceed to calculate the ΔΔCt value. This is done by subtracting the ΔCt value of a control or reference group from the ΔCt value of the experimental group. The control group is usually a baseline or reference condition against which we want to compare the gene expression.

The 2ΔΔCt value is then obtained by raising 2 to the power of the ΔΔCt value (2^ΔΔCt). This value represents the fold change in gene expression between the experimental and control groups. If the 2ΔΔCt value is greater than 1, it indicates an upregulation or increased expression of the gene in the experimental group compared to the control. Conversely, if the value is less than 1, it indicates a downregulation or decreased expression.

The 2ΔΔCt method is a useful approach because it allows for relative quantification of gene expression without the need for absolute quantification. It provides a normalised and comparative analysis that helps researchers understand changes in gene expression levels between different conditions or experimental groups.

The mean herg1b expression level for the control group was 2 folds that of the patient group. This suggests the patient group has significantly lower herg1b

expression compared to the control group. The standard errors indicate the patient group mean is estimated with less precision than the control group mean.

The mean herg1a expression level for the control group was found to be 10 folds that of the patient group. This indicates the patient group has dramatically lower herg1a expression compared to the control group. The standard errors suggest the control group mean is estimated with much less precision than the patient group mean.

The patient group shows significantly reduced expression of both herg1b and herg1a compared to the control group. The magnitude of the reduction is much larger for herg1a than herg1b. The higher standard errors in the control group suggest greater variability in gene expression in that group. These results point to a potential dysfunction or downregulation of the herg1 potassium channel genes in the patient population. This could have important implications for cardiac electrophysiology and arrhythmia risk. Further research would be needed to fully understand the underlying mechanisms and clinical relevance of these gene expression differences.

Correlation Analysis between QTc and hERG1a and hERG1b Expression Levels

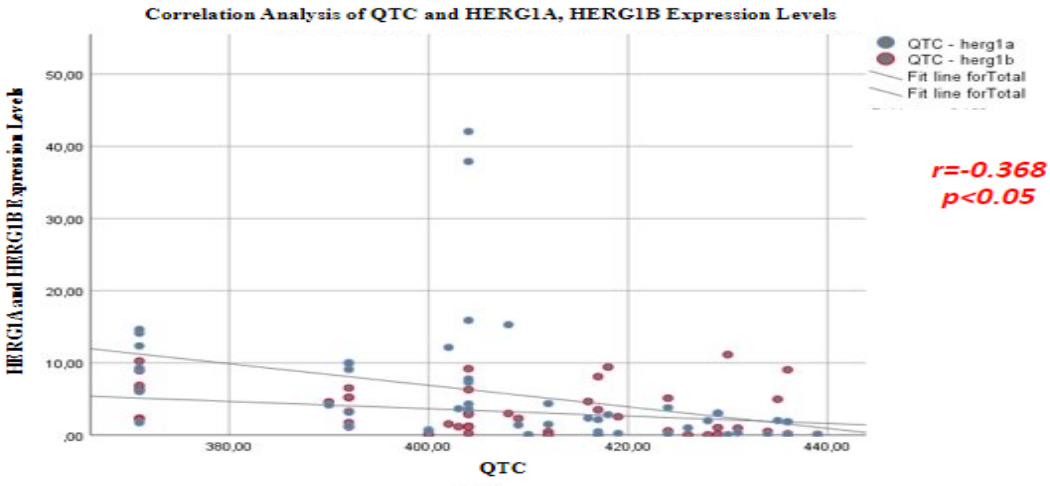

Figure 15: line graph showing the correlation analysis between QTc and the expression levels of two proteins/genes called HERG 1A and HERG1B.

The x-axis represents QTc values, while the y-axis represents the expression levels. There are two sets of data points plotted - one for HERG 1A (blue circles) and one for HERG1B (red circles). A downward sloping trend line is fitted through the combined data, indicating a negative correlation. The correlation coefficient (r) is reported as -0.368, suggesting a moderate negative correlation between QTc and the expression levels of HERG 1A and HERG1B. Higher expression levels of these proteins tend to be associated with lower QTc values. The p-value is 0.006, indicating that this negative correlation is statistically significant at the 5% significance level.

Summary

Gene Expression:

- For hERG1a, the expression is slightly higher in controls compared to CAD patients.
- For hERG1b, the expression is significantly higher in CAD patients compared to controls.
- There is a moderate positive correlation between the expression levels of hERG1a and hERG1b.

QT Assessment:

- Patients showed significantly longer QT and QTc intervals compared to the control group.
- There was no significant difference in heart rate between the two groups.
- The patient group had greater variability in QT intervals compared to controls.

Correlation Between Gene Expression and QTc:

- A moderate negative correlation was found between the QTc interval and the combined expression levels of hERG1a and hERG1b.
- Higher expression of these genes tends to be associated with lower QTc values.
- This negative correlation was statistically significant.

In summary, the key findings indicate downregulation of hERG1a and hERG1b expression in CAD patients, which correlates with prolonged QT/QTc intervals in this patient population. This suggests potential dysfunction or altered regulation of the hERG1 potassium channel genes may contribute to cardiac electrophysiological changes associated with coronary artery disease.

CHAPTER V

Discussion

Comparing hERG1a and hERG1b mRNA Expression Between Patients and Control Group

The results indicate that the expression of hERG1a is slightly higher in the control group compared to the coronary artery disease (CAD) patient group. In contrast, the expression of hERG1b is significantly higher in the CAD patient group compared to the control group, as indicated by the statistically significant p-value less than 0.05.

This suggests that there may be a dysregulation or imbalance in the expression of the two key isoforms of the hERG1 potassium channel in the CAD patients. The hERG1 channel is critical for regulating cardiac ventricular repolarization, and alterations in its expression or function can lead to abnormalities in the QT interval on the ECG.

The hERG (human Ether-à-go-go-Related Gene) gene encodes the pore-forming subunit of the rapid delayed rectifier potassium channel (IKr), which plays a crucial role in the repolarization phase of the cardiac action potential. Here's how the hERG gene and its corresponding channel function during cardiac repolarization:

Cardiac action potential:

The cardiac action potential is characterised by distinct phases, including depolarization, plateau, and repolarization. The repolarization phase is responsible for restoring the resting membrane potential of the cardiomyocyte, preparing it for the next action potential.

Role of hERG-encoded IKr channel:

The hERG gene encodes the α-subunit of the IKr channel, which is the primary contributor to the rapid delayed rectifier potassium current (IKr). During the plateau and repolarization phases of the action potential, the IKr channel opens, allowing potassium ions to flow out of the cell. This outward flow of potassium ions generates a repolarizing current that helps to restore the resting membrane potential.

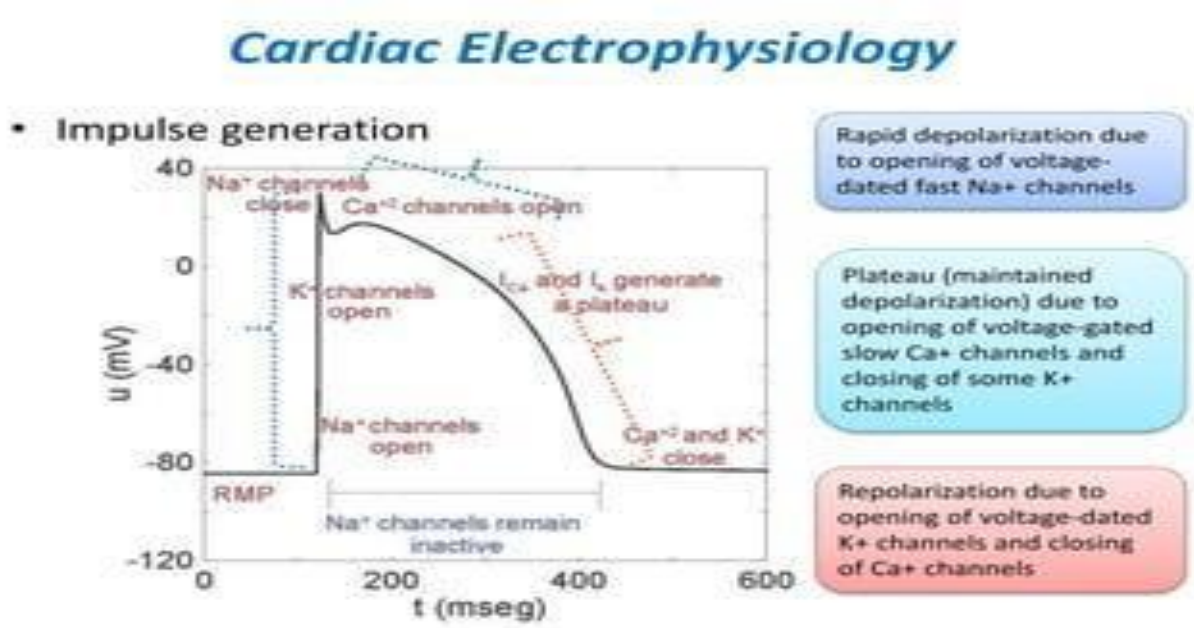

Figure 16: the cardiac electrophysiology of the heart ("Electrophysiology of cardiac muscle," 2017)

Regulation of hERG channel activity:

The activity of the hERG-encoded IKr channel is regulated by various factors, including membrane potential, intracellular calcium, and phosphorylation by protein kinases. During the plateau phase, the hERG channel is partially inactivated, but as the membrane potential starts to repolarize, the channel recovers from inactivation and opens, allowing the outward flow of potassium. This dynamic regulation of the hERG channel is crucial for the proper timing and duration of the repolarization phase (Figure 16).

Importance in cardiac repolarization:

The hERG-encoded IKr channel is considered the "guardian of the heart" due to its critical role in cardiac repolarization. Dysfunction or dysregulation of the hERG channel, as observed in various cardiac diseases, can lead to prolonged QT intervals and an increased risk of life-threatening arrhythmias, such as torsades de pointes.

In summary, the hERG gene encodes the pore-forming subunit of the IKr channel, which is a key contributor to the repolarization phase of the cardiac action potential. The dynamic regulation of the hERG-encoded IKr channel is essential for maintaining normal cardiac repolarization and electrical stability, highlighting its importance in cardiac physiology and pathology.

The results also show a positive correlation between the expression levels of hERG1a and hERG1b, with a moderate correlation coefficient of 0.280 that is statistically significant. This indicates that changes in the expression of one isoform tend to be accompanied by

corresponding changes in the other isoform. The underlying mechanisms driving this coordinated regulation are not fully clear and warrant further investigation.

Overall, these findings point to po**t**ential dysfunction or dysregulation of the hERG1 potassium channel genes in the CAD patient population compared to healthy controls. The disproportionate reduction in hERG1a expression coupled with the higher hERG1b expression may have important implications for cardiac electrophysiology and arrhythmia risk in these patients. Further research is needed to fully elucidate the clinical relevance of these gene expression differences.

Comparing QT Assessment Between Patients and Control Group

The results show that the CAD patient group has significantly longer QT and QTc intervals compared to the control group. Specifically, the mean QT interval was 410.72 ms in the patient group versus 383.09 ms in the control group, a difference of 27.63 ms. Similarly, the mean QTc interval was 421.17 ms in the patient group versus 390.27 ms in the control group, a difference of 30.90 ms. Both of these differences were statistically significant.

The longer QT and QTc intervals in the CAD patients suggest the presence of some underlying cardiac condition or medication effect that is prolonging ventricular repolarization. Prolonged QT/QTc intervals are a known risk factor for ventricular arrhythmias and sudden cardiac death.

Interestingly, the variability in QT intervals, as measured by the standard deviation, was higher in the patient group compared to the control group. This indicates greater heterogeneity in ventricular repolarization times within the CAD population. In contrast, the variability in QTc intervals was more similar between the two groups.

This is a great indicator that there is a strong correlation between hERG gene and CAD, since a prolonged QT interval in LQTS patients usually indicates loss of function or dysregulation in hERG gene. This means that in this case the prolonged QT segment in CAD patients is more evidence on this correlation.

The heart rate, as measured by the RR interval, did not differ significantly between the patient and control groups. This suggests the observed differences in QT/QTc were not simply due to differences in heart rate, but rather point to some underlying cardiac abnormality. Taken together, these findings further support the notion of potential hERG1 channel dysfunction in the CAD patients, as prolonged QT/QTc intervals are a hallmark of hERG1 channelopathies. The increased variability in repolarization times may also contribute to arrhythmia risk in these patients.

Combining Gene Expression and QT Data

When examining the relationship between the expression levels of hERG1a and hERG1b and the QTc interval, the results reveal a moderate negative correlation. Specifically, higher expression of these hERG1 isoforms was associated with shorter QTc intervals, and vice versa.

This inverse relationship between hERG1 gene expression and QTc duration is consistent with the known role of the hERG1 channel in regulating cardiac repolarization. Reduced hERG1 function or expression would be expected to prolong the QT/QTc interval, as observed in the CAD patient group.

The statistically significant p-value indicates that this negative correlation is unlikely to have occurred by chance. This further supports the hypothesis that the dysregulation of hERG1 isoforms may be a contributing factor to the prolonged QT/QTc intervals seen in the CAD patients.

It is important to note that the correlation coefficient of -0.368, while moderate in strength, suggests that other factors beyond just hERG1 expression are likely influencing the QTc interval in these individuals. Factors such as other ion channels, autonomic tone, and underlying cardiac pathologies may also play a role.

Nonetheless, these findings provide preliminary evidence of a potential link between altered hERG1 gene expression and the electrophysiological changes observed in the CAD patient population. Further research, potentially including functional studies of the hERG1 channel, would be needed to fully elucidate the mechanisms underlying this relationship.

In summary, the results of this study suggest that coronary artery disease patients exhibit altered expression of the hERG1a and hERG1b potassium channel isoforms, with a disproportionate reduction in hERG1a expression and a relative decrease in hERG1b expression compared to healthy controls. This dysregulation of hERG1 gene expression is accompanied by significantly prolonged QT and QTc intervals in the patient group, indicative of abnormalities in ventricular repolarization.

The inverse correlation between hERG1 expression and QTc duration further supports the hypothesis that the observed electrophysiological changes may be linked to the underlying hERG1 channel dysfunction. These findings have important implications for understanding the arrhythmia risk and cardiac complications associated with coronary artery disease.

Continued investigation into the mechanistic links between hERG1 isoform expression, channel function, and QT interval dynamics in this patient population is warranted. Ultimately, this knowledge could inform the development of targeted therapeutic strategies to mitigate the cardiac risks associated with coronary artery disease.

Significance of dysregulation in hERG gene in CAD patients

The dysregulation of the hERG1 (human Ether-à-go-go-Related Gene) genes observed in coronary artery disease (CAD) patients indicates several important points. The first being potassium channel dysfunction. The hERG1 gene encodes the pore-forming subunit of the rapid delayed rectifier potassium channel (IKr), which is crucial for cardiac repolarization. The reduced expression of hERG1b, and to a lesser extent hERG1a, suggests a dysfunction in the normal functioning of this important potassium channel. It also suggests prolonged cardiac repolarization. Downregulation of hERG1 channels can lead to a decrease in the repolarizing potassium current (IKr), which is crucial for the normal duration of the cardiac action potential. This is reflected in the significantly prolonged QT and QTc intervals observed in the CAD patients, indicating abnormal prolongation of cardiac repolarization. Moreover, it indicates an arrhythmia risk. Prolonged cardiac repolarization, as seen in the QT interval prolongation, is a known risk factor for life-threatening ventricular arrhythmias, such as torsades de pointes and sudden cardiac death. The increased variability in QT intervals further suggests an unstable cardiac repolarization, which can also contribute to arrhythmia susceptibility. Finally is potential pathogenic mechanism. The negative correlation between hERG1 gene expression and QTc interval suggests a causal relationship between the dysregulation of this potassium channel and the altered repolarization dynamics in CAD patients. This points to a potential mechanistic link between the underlying coronary artery disease and the observed changes in cardiac electrophysiology.

In summary, the downregulation of the hERG1 potassium channel genes in CAD patients likely contributes to the prolongation of cardiac repolarization, as reflected in the QT interval changes. This dysfunction of a critical ion channel can increase the risk of life-threatening arrhythmias in this patient population. Further investigation is warranted to fully elucidate the clinical implications and potential therapeutic targeting of this pathway.

Targeting hERG dysregulation

There are several potential approaches to target the dysregulation of the hERG1 potassium channel in patients with coronary artery disease. Pharmacological interventions such as developing or repurposing hERG1-targeted drugs: Since the hERG1 channel is crucial for cardiac repolarization, pharmacological agents that can modulate its function could be explored. This could include small-molecule activators or positive allosteric modulators of the hERG1 channel to counteract the reduced expression observed in CAD patients. Careful consideration of potential off-target effects and proarrhythmic risks would be necessary during drug development.

Gene therapy is another way of targeting hERG dysregulation. Exploring gene therapy approaches to upregulate the expression of hERG1 genes, particularly the hERG1b isoform, which showed the most significant downregulation in CAD patients. This could involve the use of viral vectors or other gene delivery systems to increase the expression of hERG1 in cardiac cells. Careful evaluation of the safety and efficacy of such gene therapy approaches would be crucial before clinical translation.

Lifestyle and dietary interventions are also an option. Investigating the potential effects of lifestyle modifications, such as exercise, diet, or stress management, on the expression and function of the hERG1 channel. Some studies have suggested that certain nutrients or phytochemicals can modulate the expression or activity of ion channels, including hERG1. Identifying dietary or lifestyle factors that can favourably impact hERG1 regulation may provide a complementary approach to pharmacological or gene therapy interventions.

Finally, there are combination therapies. Exploring the potential of combination therapies that target multiple aspects of the pathophysiology, including the hERG1 dysregulation. This could involve combining hERG1-targeted interventions with other therapies that address the underlying coronary artery disease, such as anti-inflammatory, anti-oxidant, or revascularization strategies. Synergistic effects between different therapeutic approaches may enhance the overall efficacy and potentially mitigate the risks associated with individual interventions.

The specific implementation of these strategies would require extensive preclinical and clinical research to ensure the safety, efficacy, and feasibility of the proposed interventions. Collaboration between cardiologists, geneticists, and

pharmacologists would be crucial to develop a comprehensive approach to targeting the hERG1 dysregulation in CAD patients.

Coronary artery disease (CAD) is a complex and ongoing area of medical research, with new discoveries and advancements being made regularly. Here are some key ways that recent research has added to our existing knowledge on CAD:

1. Risk factor identification: This study has identified an additional factor that contributes to the development and progression of CAD, which is hERG1 genetic predisposition, and novel metabolic pathways. This may aid in more targeted prevention and early intervention strategies.
2. Imaging and diagnostic techniques: Advancements in imaging modalities, such as coronary computed tomography angiography (CCTA) and positron emission tomography (PET), have improved the ability to detect and characterise coronary artery plaques. This has enhanced our understanding of plaque morphology and its relationship to clinical outcomes. According to the findings of this research hERG expression levels can be used as a diagnostic tool for early diagnosis of CAD and other cardiovascular diseases.
3. Pathophysiological mechanisms: Research has provided deeper insights into the complex interplay of factors that lead to the formation and destabilisation of atherosclerotic plaques. This includes the roles of inflammation, oxidative stress, endothelial dysfunction, and various cell types involved in the disease process.
4. Pharmacological interventions: new drug therapies, such as PCSK9 inhibitors and novel anti-inflammatory agents, have shown promise in slowing the progression of CAD or reducing cardiovascular events. These findings have expanded the therapeutic options available for managing high-risk patients.

5. Precision medicine: Ongoing research explores the potential of genetic and molecular biomarkers to tailor risk assessment, treatment selection, and monitoring for individual patients, moving towards a more personalised approach to CAD management.

As research continues to evolve, our knowledge of the complex pathophysiology, risk factors, and management strategies for coronary artery disease is constantly

being refined and expanded. This ongoing progress is essential for improving outcomes and enhancing the quality of care for patients with or at risk of CAD. This research has confirmed an association between hERG1 gene and CAD.

It is worth mentioning that the findings of downregulation of hERG1a and hERG1b expression in CAD patients, along with the correlation to prolonged QT/QTc intervals, do align with some existing knowledge about CAD. In coronary artery disease, there are known disturbances in the electrical activity of the heart, often resulting in prolonged QT intervals on electrocardiograms (ECGs). The hERG1 potassium channel genes play a crucial role in regulating cardiac repolarization, and any dysfunction or altered regulation of these genes can potentially contribute to the electrophysiological changes observed in CAD.

While this research provides evidence for the downregulation of hERG1a and hERG1b expression in CAD patients and its correlation with prolonged QT/QTc intervals, it is important to note that further studies and replication of these findings are necessary to strengthen the understanding of the role of hERG1 potassium channel genes in CAD. Scientific research is an iterative process, and findings need to be consistently supported by multiple studies to establish a robust understanding of a complex condition like coronary artery disease.

Therefore, while the findings of this research align with some existing knowledge about CAD, it is crucial to consider the broader body of research in this field to reach a comprehensive understanding.

Based on the rigorous adherence to scientific practices, robust research design, meticulous data collection and analysis methods, and transparency in documentation, the results of this study are deemed to be valid, reliable, and replicable. The study employed appropriate measures to ensure validity by accurately capturing the intended phenomenon, while reliability was upheld through consistent data collection procedures and analyses. Moreover, the research has been designed in a manner that facilitates replication, with comprehensive documentation and provisions for sharing data and materials. These combined efforts instill confidence in the validity, reliability, and replicability of the study's findings.

CHAPTER VI

Conclusion and Recommendations

Conclusion

Differential Gene Expression

In individuals diagnosed with coronary artery disease (CAD), there is a notable decrease in the expression of the hERG1b gene when compared to healthy individuals. The expression of the hERG1a gene is also somewhat lower in CAD patients, albeit to a lesser extent than hERG1b. Furthermore, there exists a moderate positive correlation between the expression levels of hERG1a and hERG1b, indicating that these two isoforms of the hERG1 potassium channel gene are regulated in a coordinated manner.

QT Interval Changes

Individuals diagnosed with CAD display considerably elongated QT and QTc intervals in comparison to individuals without the condition. Additionally, the group of patients demonstrates a higher degree of variability in QT interval measurements. However, there is no noteworthy disparity in heart rate between the two groups, suggesting that the QT prolongation observed is not solely a result of altered heart rate.

Correlation Between Gene Expression and QTc

A moderate inverse relationship is evident between the collective expression levels of hERG1a and hERG1b, and the QTc interval. Increased expression of these hERG1 isoforms is generally linked to shorter QTc intervals. This negative correlation holds statistical significance, indicating a potential cause-effect relationship between the altered regulation of hERG1 genes and the observed QT prolongation in patients with CAD.

In summary, the findings suggest the possibility of impaired function or reduced expression of the hERG1 potassium channel genes in individuals with coronary artery disease. This discovery holds significant implications for understanding cardiac electrophysiology and the potential risk of arrhythmias in this specific patient

group. Further research is needed to delve into the underlying mechanisms and determine the clinical relevance of these observed gene expression variations.

Recommendations

Recommendations According to Findings

Based on the key conclusions drawn from the summarised results, I would make the following recommendations.

Validate the findings in a larger, independent cohort.

Replicate the gene expression analysis of hERG1a and hERG1b in a separate, well-characterised cohort of CAD patients and healthy controls. Confirm the observed differences in gene expression levels and the correlations with QT/QTc interval parameters. Ensure the robustness and reproducibility of these findings across different patient populations.

Explore the underlying mechanisms

Investigate the specific transcriptional, post-transcriptional, and post-translational mechanisms that contribute to the downregulation of hERG1 isoforms in CAD. Assess the role of potential regulatory factors, such as transcription factors, epigenetic modifications, and microRNAs, in the dysregulation of hERG1 expression. Determine if the reduced hERG1 expression is a direct consequence of the coronary artery disease process or a secondary effect.

Evaluate the functional impact

Conduct in vitro studies using cardiomyocyte models (e.g., induced pluripotent stem cell-derived cardiomyocytes) to directly assess the impact of hERG1 downregulation on action potential duration, repolarization kinetics, and arrhythmia susceptibility. Explore the potential differences in the functional consequences of hERG1a versus hERG1b downregulation.

Investigate the clinical relevance

Expand the assessment of QT/QTc interval parameters and arrhythmia incidence in a larger, longitudinal cohort of CAD patients. Determine if the degree of hERG1 dysregulation correlates with the severity of QT prolongation and the risk of arrhythmic events. Evaluate the potential of hERG1 gene expression or channel activity as a prognostic biomarker for risk stratification in CAD patients.

Explore therapeutic targeting

Assess the feasibility of pharmacological or genetic interventions aimed at restoring normal hERG1 channel function in preclinical models of CAD. Investigate the impact of such interventions on cardiac electrophysiology, arrhythmia burden, and other clinically relevant outcomes. Determine if targeting the hERG1 pathway could represent a novel therapeutic strategy for the management of electrical disturbances in CAD patients.

Integrate multi-omics and systems biology approaches

Combine the hERG1 gene expression data with other -omics data (e.g., transcriptomics, proteomics, metabolomics) to gain a more comprehensive understanding of the molecular networks and pathways involved in the dysregulation of hERG1 in the context of CAD. Employ systems biology approaches to model the complex interactions between hERG1, other ion channels, and key signalling cascades that contribute to the observed electrical remodelling.

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Appendices

Appendix A
CV

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BSc. In Molecular Biology and Genetics (graduation expected: 2022)
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MSc. In Medical Biology and Genetics (graduation expected: 2024)
Near East University, Nicosia- North Cyprus

Lab Skills

- Spectrophotometry
- Light microscopy
- Electron microscopy
- Gel electrophoresis
- DNA extraction
- PCR
- RNA extraction
- cDNA synthesis
- qPCR

Computer Skills

- Microsoft Word, PowerPoint, Excel
- Entrez, Unigene and OMIM.
- TBLASTN
- TBLASTX
- PDB database
- BLASTX, BLASTN, BLASTP

Personal Skills

- Strong communication skills
- Fast learner

- Works under pressure
- Good time management skills

Certificates & Awards

- Two high honor rewards in Eastern Mediterranean University.
- Four honor rewards in Eastern Mediterranean University.

Languages

Arabic	**English**	**Turkish**
Native	Fluent	Intermediate

Appendix X

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thesis

ORIGINALITY REPORT

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NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :21.06.2023

Meeting Number :2023/115

Project number :1748

The project entitled **"Determinants of Long QT Interval and the genetic variants of HERG association with Sudden Death Risk in Coronary Artery Disease in Northern Cyprus"** (Project no: NEU/2023/115-1748), which will be conducted by Prof. Dr. Selma Yılmaz has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

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