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Detection of Familial Hypercholesterolemia Variants in Selected Patients with Premature Coronary Artery Disease from Hebron Region

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Thesis Approval

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Dedication

First and foremost, I want to praise the Almighty God for completing my thesis Alhamdulillah.

This work is wholeheartedly dedicated to my backbone; my beloved husband, who has been constantly by my side. To my father's soul who spent his life teaching me how to fly high. To my mother, that kind solid woman. To sisters and brothers. To my father, mother, sisters and brothers in low to hold us and our daughters while being busy. To my friends, friends that never let me down. Last but not least, to my two little pieces of Swiss chocolate: my daughters Elaf and Hoor.

I dedicate my thesis to the experiences I never expected and the paths that were redirected.

1445-2024

Declaration

I certify that this thesis submitted for the degree of master's is the result of our research; the content of the thesis is the result of work that has been carried out since the date of approval of the research program. All ethics procedures and guidelines have been appropriately followed while preparing the thesis.

Signed:

Enas Yousef Mustafa Sarahna

Date: 15 / 05 /2024

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Finally, I’m extremely grateful to the family who participated in this research and I hope this work will improve their health outcome.

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Abstract

Background:

The Familial hypercholesterolemia disorder is prevalent but varies across ethnicities. Familial hypercholesterolemia is an autosomal dominant disease with 87% penetrance caused by pathogenic variants in the genes involved in cholesterol metabolism: LDL Receptor, apolipoprotein B or Proprotein Convertase Subtilisin/ Kexin 9 genes, resulting in impaired clearance of circulating Low Density Lipoprotein cholesterol, given that up to 90% of genetically confirmed familial hypercholesterolemia have LDL Receptor variants. It is a highly atherogenic metabolic disorder characterized by lifelong vascular exposure to LDL-C, leading to premature coronary artery disease.

Purpose: This work aims to identify the pathogenic variant that cause this disease and verify cascade screening among the studied family.

Methods: We selected a patient who fulfilled our inclusion criteria: (1) fasting plasma LDL-C level >190 mg/dL and triglycerides < 220 mg/dL; (2) presence of tendon xanthomata/xanthelasma/corneal arcus or premature coronary artery disease or a first degree relative, or a family history of hypercholesterolemia. The proband's whole blood sample was sent to Whole Exome Sequencing. Then, blood samples were drawn for PCR and Sanger sequencing from his first-degree relatives. Any newfound case was treated as a new index, and his/her first-degree relatives were screened for that variant.

Results: Findings showed that our proband has a heterozygous likely pathogenic missense NM_000527.2: c.1210A>G p. (Thr404Ala) variant in exon 9 of LDL Receptor gene. This variant has not yet been submitted to the Clinvar database. Screening results of his first-degree relatives showed that this variant was transmitted from his father (homozygous familial hypercholesterolemia: GG) to all of his brothers (heterozygous familial hypercholesterolemia: AG). First-degree relatives of affected individuals were screened; a pedigree was drawn. Sequence result and LDL-C level was significantly correlated.

Conclusion: Familial hypercholesterolemia is underdiagnosed and undertreated in our population, and this increased the number of premature Atherosclerotic cardiovascular disease cases. Cascade screening is a beneficial and cost-effective process for the diagnosis and treatment of familial hypercholesterolemia early in life using lipid-lowering agents in order to decrease the burden of Atherosclerotic cardiovascular disease and prevent premature cardiovascular death among our population. The variant NM_000527.2: c.1210A>G p. (Thr404Ala) is highly suspected to be FH-causing as A>G substitution has a significant correlation with LDL-C mean level.

Keyword: Familial hypercholesterolemia, LDL Receptor, atherosclerotic cardiovascular disease, cascade screening, whole exome sequencing.

TABLE OF CONTENTS

List of figures

List of tables

List of Abbreviation

Acronym	Full Form
FH	Familial Hypercholesterolemia
LDL-C	Low Density Lipoprotein Cholesterol
HDL-C	High Density Lipoprotein”
VLDL-C	Very Low Density Lipoprotein”
HeFH	Heterozygous Familial Hypercholesterolemia
HoFH	Homozygous Familial Hypercholesterolemia
LDLR	Low Density Lipoprotein Receptor
PCSK9	Proprotein Convertase Subtilisin/Kexin 9
ApoB	Apolipoprotein B
CAD	Coronary Artery Disease
HMG-CoA	Hydroxy Methylgluteryl-Coa
SREBPs	Sterol Regulatory Element-Binding Proteins
ER	Endoplasmic Reticulum
MVA	Mevalonate
NPC1L1	Niemann-Pick C1-Like 1

Acronym	Full Form
ACAT	Acyl-Coenzyme A Cholesterol Acyltransferase
FFAs	Free Fatty Acids
LRPs	LDLR Related Proteins
ABCG1	Atp-Binding Cassette Subfamily G Member 1
LCAT	Lecithin-Cholesterol Acyltransferase
MTP	Microsomal Triglyceride Transfer Protein
COPII	Coat Protein Complex II
CM	Chylomicrons
LPL	Lipoprotein Lipases
TG	Triglycerides
CVD	Cardiovascular Disease
ASCVD	Atherosclerotic Cardiovascular Disease
LDLRAP1	Low Density Lipoprotein Receptor Adaptor Protein 1
PRS	Polygenic Risk Score
SNPs	Single Nucleotide Polymorphisms
SCAP	SREBP Cleavage Activating Protein

Acronym	Full Form
SRE	Sterol Regulatory Element
GOF	Gain Of Function
LOF	Loss Of Function
DLCN	Dutch Lipid Clinical Network Criteria
NGS	Next Generation Sequencing
APEX	Arrayed Primer Extension
ESC	European Society of Cardiology
ROS	Reactive Oxygen Species
AGEs	Advanced Glycated End-Products
VSMCs	Vascular Smooth Muscle Cells Are
ECM	Extra Cellular Matrix
PAD	Peripheral Arterial Disease
CKD	Chronic Kidney Disease
AoVC	Aortic Valve Calcification
AVS	Aortic Valve Stenosis
Lp(a)	Lipoprotein-A

Acronym	Full Form
AVR	Aortic Valve Replacement
cIMT	Carotid Intima-Media Thickness
CAC	Coronary Artery Calcium Scoring
CCTA	Coronary Computed Tomography Angiography
MENA	Middle-East and North Africa
WES	Whole Exome Sequencing
CNVs	Copy Number Variations
PCR	Polymerase Chain Reaction
AMI	Acute Myocardial Infarction
FFR	Fractional Flow Reserve
MACE	Major Adverse Cardiac Events

CHAPTER 1
(INTRODUCTION)

1.1 Background

Cholesterol is an essential cell membrane component that regulates fluidity and interaction with proteins and lipids. It serves as a precursor for various essential molecules, including steroid molecules, bile salts, steroid hormones and vitamins. Cholesterol has complex functions that extend as a precursor and participate in various metabolic pathways, which require strict regulation to achieve cholesterol homeostasis (Benito-Vicente et al., 2018).

Familial hypercholesterolemia (FH) is an autosomal dominant disease mainly caused by pathogenic variants in cholesterol metabolism responsible genes, which cause buildup of Low Density Lipoprotein cholesterol (LDL-C) in plasma due to impaired clearance. It is a highly atherogenic metabolic disorder characterized by lifelong vascular exposure to LDL-C, leading to premature coronary artery disease (Chemello et al., 2021). The estimated FH prevalence varies across ethnicities, ranging from 1:400 to 1:192, with the highest prevalence among black and brown and the lowest among Asian individuals (Toft-Nielsen, Emanuelsson, & Benn, 2022). The overall prevalence of FH in the Arabian Gulf region is estimated to be 1/232, while prevalence in other Arabian countries is not yet estimated (Awan et al., 2021).

FH typically shows autosomal dominant mode of inheritance with either heterozygous (HeFH) or homozygous (HoFH) genotypes based on one or two copies of pathogenic variants in LDL Receptor (*LDLR*), apolipoprotein B (*APOB*) or proprotein convertase subtilisin/ kexin 9 (*PCSK9*) genes. Most clinically diagnosed FH patients have a mono-allelic loss of function variants in the LDLR gene, responsible for 85% to 90% of genetically confirmed FH. Pathogenic variants of the apolipoprotein (ApoB) gene, resulting in decreased binding of LDL-C to the LDLR caused by conformational changes in apoB or gain-of-function mutations in the gene for Proprotein Convertase Subtilisin/Kexin 9 (PCSK9); resulting in increased destruction of LDL-C-R; are responsible for 5% to 15% and 1% of cases of FH, respectively (McGowan, Hosseini Dehkordi, Moriarty, & Duell, 2019). Pathogenic variants in one of these genes (monogenic) can be found in about 80% of clinically diagnosed FH patients, whereas the other 20% were the result of a

polygenic cause, which implies the importance of differentiating the underlying cause of hypercholesterolemia (Tandirerung, 2022).

Polygenic hypercholesterolemia occurs in patients clinically diagnosed with FH when established *LDLR, APOB*, or *PCSK9* mutations are not recognized. The involvement of multiple genes is assumed to be the cause and is referred to as polygenic hypercholesterolemia in the absence of monogenic mutations (Tandirerung, 2022). Although molecular diagnosis is available, it is not yet the first-line tool used. So, clinical diagnostic criteria have been set to recognize highly susceptible patients with FH. The most familiar criteria are the Simon-Broome Criteria and The Dutch Criteria. The latter, Dutch criteria, is a modified version of the Simon-Broome criteria. Evidence of family history of hyperlipidemia or heart disease, along with specific clinical characteristics, including tendinous xanthomata, elevated LDL-C cholesterol, and/or an identified mutation, are points of consideration within the Dutch criteria. A total score of more than eight is treated as "definite" FH, 6–8 as "probable" FH and 3–5 as "possible" FH (Al-Rasadi et al., 2014).

Recent studies have shown that common mutations in the Middle East and North Africa (MENA region) were in *LDLR* and *PCSK9* genes but not in *APOB* (Baltimore et al., 2015), whereas in Israel, most of the mutations were found to be in the *LDLR* and *APOB* genes (Durst et al., 2017).

People with monogenic FH are at a greater risk of developing premature coronary artery disease (CAD before age 45 years in men and 55 years in women), peripheral arterial disease and chronic kidney disease. At the same time, the polygenic cause was not different compared to patients without genetic cause of hypercholesterolemia. Also, patients with FH who underwent coronary angiography due to CAD needed more revascularization sessions than the general population after a 10-year follow-up period (Trinder et al., 2019).

The underdiagnosis and undertreatment of FH are still a challenge. It has been reported that clinical criteria for FH diagnosis are missing around half the cases, so a genetic diagnosis should be performed. As mentioned before, a good proportion of hypercholesterolemia is due to polygenic causes, so proper selection of patients and proper genetic diagnosis could improve the identification of FH and referral to specialist assessment. Early diagnosis of FH and identification of the underlying pathogenic variants should reduce the risk of arterial disease.

Treatment of FH should be directed towards the exact pathogenic variant that exists in each patient. Initiation of statin (HMG-CoA reductase inhibitors) treatment during childhood delayed Carotid Intima/Medi thickness progression in patients with familial hypercholesterolemia and reduced the risk of cardiovascular disease in adulthood (Luirink et al., 2019). In PCSK9 gain of function mutation, PCSK9 inhibitors (Alirocumab and Evolocumab) are used. Alirocumab resulted in a significant and clinically observed reduction in LDL-C levels, which was well-tolerated among those patients (Blom et al., 2020). Trials are established to design a gene therapy module for FH. In a mouse model, exosome-mediated LDLR mRNA delivery effectively restored the expression of the receptor (Li et al., 2021). This appears to be a promising therapeutic tool that will treat FH and reduce the risk of atherosclerosis.

1.2 Problem statement

In Palestine, FH represents a significant and under-recognized public health challenge, characterized by undetermined prevalence and pathogenic variants, lack of systemic screening, limited access to specialized healthcare, and inadequate awareness, which collectively contribute to the underdiagnosis and suboptimal management of this genetic disorder. Many patients admitted to Palestinian hospitals with premature CAD have impaired serum lipid profiles, mainly high LDL-C serum levels, and most of them have a strong family history of both premature CAD and high LDL-C levels. This situation results in a high burden of cardiovascular disease and poses a substantial threat to the well-being of affected individuals and their families in Palestine.

This Problem statement underscores the pressing issue related to FH in Palestine, including the need for improved screening and diagnostic strategies, greater access to appropriate medical care, and enhanced public and healthcare provider awareness to effectively address this genetic condition and reduce its associated cardiovascular risks in the Palestinian population.

1.3 Study hypothesis

Patients with premature CAD and high LDL-C levels have a genetic variant of FH.

1.4 Study main aim

To clarify if selected patients with premature coronary artery disease and high LDL-C levels from Hebron region have familial hypercholesterolemia as a primary cause.

1.5 Study objectives

- To verify the concept of cascade screening for FH in families that have members with premature CAD and elevated plasma LDL-C.
- To spot the light on the causes of premature coronary artery disease.
- To clarify if FH is the cause of premature coronary artery disease.
- To identify pathogenic variants that cause FH in selected Palestinian patients from Hebron region.

CHAPTER TWO
(LITERATURE REVIEW)

2.1 Cholesterol

Cholesterol was extracted from bile stones for the first time in 1789 and has since been extensively explored. It is a crucial component of the cell membrane that regulates fluidity and interacts with proteins and lipids. It also serves as a precursor for various steroid compounds, including bile salts, steroid hormones, and vitamins. Cholesterol has complicated activities as a precursor and in metabolic pathways, necessitating rigorous control to achieve cholesterol homeostasis (Benito-Vicente et al., 2018).

2.1.1Cholesterol metabolism

The metabolism of cholesterol is a tightly regulated process. Cell cholesterol is synthesized or absorbed. De novo synthesis is strictly regulated, with the liver producing the vast majority of the body's cholesterol. Several proteins are essential for different people's requirements. When intracellular cholesterol levels fall below the physiologic threshold, Sterol Regulatory Element-Binding Proteins (SREBPs) in the Endoplasmic Reticulum (ER) are activated, promoting HMG-CoA reductase transcription (the rate limiting enzyme of the synthesis process of cholesterol) and activating the Mevalonate (MVA) pathway to increase intracellular cholesterol production. The Endoplasmic Reticulum (ER) synthesizes cholesterol in 19 stages before releasing it to the cytoplasm for storage as cholesterol esters or distribution (Benito-Vicente et al., 2018).

Dietary cholesterol absorption is the second major source of cholesterol. The gut absorbs cholesterol, triglycerides, and free fatty acids. Bile acids emulsify cholesterol, forming micelles of bile acid-cholesterol that are transported to the gut, where intestinal lipases release cholesterol from cholesterol esters, which is then absorbed by enterocytes via the Niemann Pick C1 like 1 (NPC1L1) protein. When free cholesterol is internalized, it is delivered back to the intestinal lumen by sterolins or re-esterified by acyl-coenzyme A cholesterol acyltransferase (ACAT). Cholesterol that underwent re-esterification can be retained in lipid droplets or straightaway packed in

triglyceride-rich chylomicrons (ApoB48 lipoproteins). The lymphatic system transports newly generated chylomicrons to the thoracic duct, which are secreted into the circulation via the left subclavian vein. Chylomicron ApoC-II stimulates lipoprotein lipases in peripheral tissues, primarily adipose and muscle tissues, resulting in triglyceride hydrolysis. Adipocytes and muscle cells then absorb free fatty acids. When FFAs from chylomicrons are hydrolyzed, they form smaller particles richer in cholesterol esters, which transmit ApoA and ApoC to other lipoproteins (mainly HDL) and acquire ApoE. Finally, ApoE interaction with LDLR and other LDLR-Related Proteins (LRPs) causes chylomicron remnants to be removed from the plasma by the liver (Benito-Vicente et al., 2018). Figure (1) represents a summary of cholesterol metabolism.

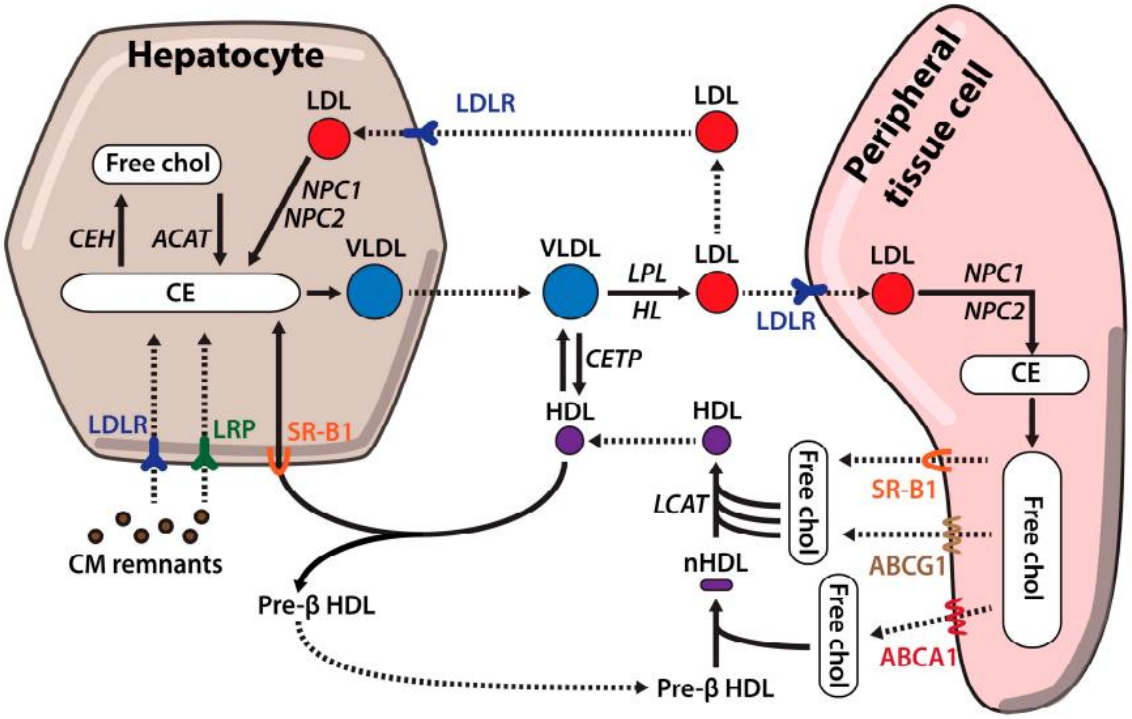


Figure (1): Cholesterol Metabolism, adapted from *(Benito-Vicente et al., 2018)*

Abbreviations: NPC1: Niemann-Pick C1; NPC2: Niemann-Pick C2; ACAT: Acyl-CoA acyl-transferase; CM: Chylomicrons; LPL: lipoprotein lipases; TG: Triglycerides; LDLR: Low Density Lipoprotein receptor; LRPs: LDLR related proteins; VLDL-C: very Low Density Lipoprotein s; HDL: High Density Lipoprotein s; ABCG1: ATP-binding cassette subfamily G member 1; LCAT: Lecithin-cholesterol acyltransferase; HDL: High density lipoproteins.

2.1.2 Hepatic cholesterol efflux and influx

The liver is the vital organ in charge of maintaining cholesterol homeostasis. It secretes cholesterol in the form of lipoproteins known as very Low Density Lipoprotein s (VLDL-C). VLDL-C is manufactured in two steps. This process begins with the translocation of nascent apoB100 across the hepatocytes' ER membrane, which is lipidated by microsomal triglyceride transfer protein (MTP). The apolipoprotein is degraded if ApoB100 is not effectively lipidated due to low triglyceride concentrations or a process failure. In a subsequent stage, vesicles containing coat protein complex II (COPII) convey partly lipidated VLDL-C particles to the Golgi. They are further lapidated in the Golgi after acquiring apoA1 and apoE apolipoproteins. Finally, mature VLDL-C particles are released into the circulation, carrying lipids to peripheral tissues. High VLDL-C excretion leads to high LDL-C blood levels, whereas insufficient VLDL-C secretion results in hepatic lipid buildup, which might be the initial step toward having fatty liver (Benito-Vicente et al., 2018).

Triglycerides from VLDL-C are removed from the plasma by the action of LPLs, resulting in VLDL-C leftovers known as intermediate-density lipoproteins (IDL). Additional IDL processing by hepatic lipases (HL) along with lipid and apolipoprotein exchange with HDL results in LDL-C formation. LDL-Cs are the body's primary cholesterol transporters, mostly made of cholesterol esters and apoB-100. They transport cholesterol from the liver to the peripheral tissues, where it binds to LDLR and is endocytosed in clathrin-coated pits. The LDLR-LDL-C complex is transported to the endocytic compartment, where it dissociates from LDL-C owing to pH acidification and is recycled back to the membrane via a pH-dependent conformational change. While LDL-Cholesterol is hydrolyzed in the lysosome due to lysosomal lipase activity to liberate free cholesterol, dissociating LDL-C from LDLR in the endosome is a critical mechanism that facilitates receptor recycling. Finally, Niemann-Pick type C1/C2 (NPC1/NPC2) proteins transport free cholesterol from lysosomes to the ER(Benito-Vicente et al., 2018).

LDLR-mediated cholesterol uptake is a highly regulated process. On the transcriptional level, SREBP-2 is inhibited when cells are rich in cholesterol or its derivatives, thereby suppressing the transcription of the *LDLR* gene or other genes necessary for lipid synthesis. On the other hand,

PCSK9 and IDOL regulate LDLR at the membrane level by interrupting LDLR recycling and promoting its degradation by preventing dissociation from LDL-C inside the endosome and being transported as a whole complex to the lysosome for degradation (Benito-Vicente et al., 2018).

To summarize, cholesterol metabolism is a complicated process that needs tight regulation. Any defect in absorption, lipoprotein production and transport, cholesterol synthesis and excretion, or cholesterol uptake by peripheral tissues can lead to a lipid metabolism disorder. Mutations that disturb LDL-C metabolism are most commonly leading to a cholesterol metabolism-derived disease known as familial hypercholesterolemia (Benito-Vicente et al., 2018).

2.2 Familial hypercholesterolemia (FH)

2.2.1 Overview

FH is an autosomal dominant disorder characterized by elevated plasma levels of LDL-C above the 95th percentile (190mg/dL) due to its reduced catabolism. As a consequence, chance of early onset atherosclerosis is increased that results in cardiovascular complications (Jarauta, Bea-Sanz, Marco-Benedi, & Lamiquiz-Moneo, 2020). Clinical FH was first described in 1937, although it was reported that single cases had hypercholesterolemia, xanthomatosis and CVD as early as 1873. In 1973, Brown and Goldstein published that FH was caused by defects in the gene coding for LDLR, thus decreasing LDL-C removal from the circulation. They were awarded the Nobel Prize for their work in 1985. Other gene defects are known to cause FH, such as *ApoB100*, *PCSK9* and LDLR adaptor protein 1 (*LDLRAP1)*. However, only 80% of clinically diagnosed FH cases were attributed to monogenic (one variant in one gene) FH-causing variants, while the remaining 20% were assigned to several LDLRaising single nucleotide variants, so-called polygenic FH (Schmidt, Hedegaard, & Retterstol, 2020).

Monogenic FH is inherited in an autosomal dominant manner with a penetrance rate exceeds 87.5%. Autosomal dominant FH could be present in a heterozygous (one allele is affected in one gene), homozygous (both alleles are affected with the same mutation in one gene), compound heterozygous (two different mutations on the two alleles in one gene) or double heterozygous (two different mutations in two different genes) genotypes in *LDLR, ApoB100 and PCSK9* genes. Also,

in rare cases, monogenic FH could be inherited in an autosomal recessive form and caused by a homozygous mutation in the *LDLRAP1* gene (McGowan et al., 2019). Here, we only reviewed the autosomal dominant FH.

On the other hand, when known FH-causing mutations are not identified (*LDLR, ApoB100, PCSK9 and LDLRAP1*) in a patient with hypercholesterolemia, incorporation of multiple other gene variants is outweighed by the cause of hypercholesterolemia, and this is called polygenic hypercholesterolemia. It is confirmed by using a polygenic risk score (PRS), which represents the likelihood of having a disease based on the genetic variants in the genome that may be associated with that disease. It calculates the aggregates of the LDLRaising effect of common variants. The higher the PRS with elevated LDL-C, the higher the possibility of having polygenic hypercholesterolemia. In comparison, patients with low PRS are considered to have monogenic FH. The more SNPs that raise LDL-C, the more severe phenotypes can be observed (Tandirerung, 2022).

Differentiating between polygenic FH and monogenic hypercholesterolemia has a clinical significance. Recent studies showed that FH caused by monogenic variations had a more elevated mean LDL-C baseline than hypercholesterolemia due to polygenic causes (293.9 mg/ dl vs. 239.75 mg/dl). Also, monogenic FH increases the risk of premature CAD by 3.5 folds, while polygenic hypercholesterolemia has a similar risk to hypercholesterolemia with no detected LDL-CC-raising variants (Tandirerung, 2022).

HeFH patients have one mutated allele, and their plasma LDL-C levels are double the normal or higher (>190mg/dL). Usually, those patients experience the first cardiovascular complications in the third decade of life. The prevalence of HeFH worldwide is about 1:250. On the other hand, in the severe form of FH, HoFH patients have mutations in both alleles, and their plasma LDL-C levels are twice those of HeFH (>500mg/dL). They develop cardiovascular complications as early as the first decade of their lives. Fortunately, HoFH is rare and has a worldwide prevalence of 1:160000-300000 (Vrablik et al., 2020).

2.2.2 FH prevalence among ethnicities

The adoption of distinct diagnostic criteria, screening techniques, and founder effects all contribute to the variation in the prevalence of FH among nations. Another huge contributing factor is

different ethnicities. A systemic review and meta-analysis study was conducted in 2022 and revealed that the highest prevalence of FH was among blacks (1:192) followed by browns (1:208), Latinos (1;280), whites (1:323) and the lowest prevalence was among Asians (1:400) (Toft-Nielsen et al., 2022).

2.2.3 Genetic background of monogenic FH

2.2.3.1 LDLR gene

The Low Density Lipoprotein Receptor (*LDLR*) gene is located on chromosome 19 short arm (19p 13.2) and has 18 exons. This gene produces a premature protein consists of 860 amino acids. The first 21 amino acids are a characteristic hydrophobic signal sequence that is cleaved off the protein before it appears on the cell surface, resulting in an 839-amino-acid mature protein with five distinct domains, which is one member of the LDLR family that orchestrates cholesterol homeostasis (see figure 2). The first domain represents the binding site of apoB and E of LDL-C and related lipoproteins, consisting of 300 amino acids rich in cysteine residues involved in disulfide bonds (Sudhof, Goldstein, Brown, & Russell, 1985). The second domain is a sequence of 400 amino acids that resembles the precursor for mouse EGF, and it plays a pivotal role in binding PCSK9 to LDLR, thus controlling lipoprotein release and receptor recycling (Galicia-Garcia et al., 2020). Domain number three consists of 48 amino acids, including 18 serines and threonines, many of which appear to serve as attachment sites for O-linked carbohydrate chains. Domain number four is a 22-amino-acid hydrophobic membrane-spanning region, whereas domain number five is a 50-amino-acid COOH-terminal cytoplasmic tail (Sudhof et al., 1985).

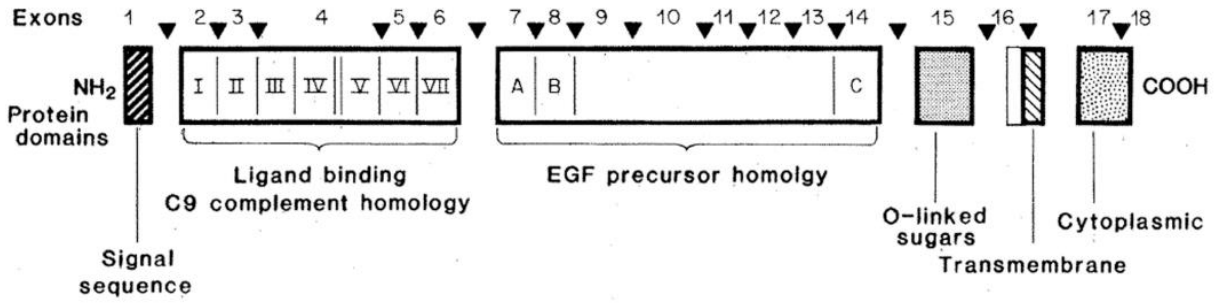


Figure (2): Exon organization and protein domains in the human LDL Receptor, adapted from (Sudhof et al., 1985). The Roman numerals refer to the LDL-C binding domain's seven cysteine-rich, 40-amino acid repetitions. A, B, and C are the three cysteine-rich repetitions in the EGF Precursor Homology Domain.

LDLR is a glycoprotein that binds to and internalizes cholesterol, mainly containing LDL-C and VLDL-C particles. It is the leading player in cholesterol homeostasis. Eliminating LDL-C from circulation begins with LDLR recognition of apoB100 on the LDL-C surface, and binding occurs as a consequence. Once the receptor-ligand complex is formed, it is endocytosed via clathrin-coated pits and sent to the endosome with Low Density Lipoprotein -related protein 6 (LRP6) and LDLRAP1. The acidic conditions inside the endosome promote receptor-ligand dissociation. The released LDL-C is distributed to the lysosome, while LDLR is recycled again to the cell surface to function and eliminate another LDL-C particle. Any error through this process could lead to the accumulation of LDL-C in the circulation and increase the risk of ASCVD (Benito-Vicente et al., 2018).

In order to achieve cholesterol homeostasis, LDLR expression is highly regulated at both transcriptional and post-transcriptional levels. Figure (3) clarifies this process.

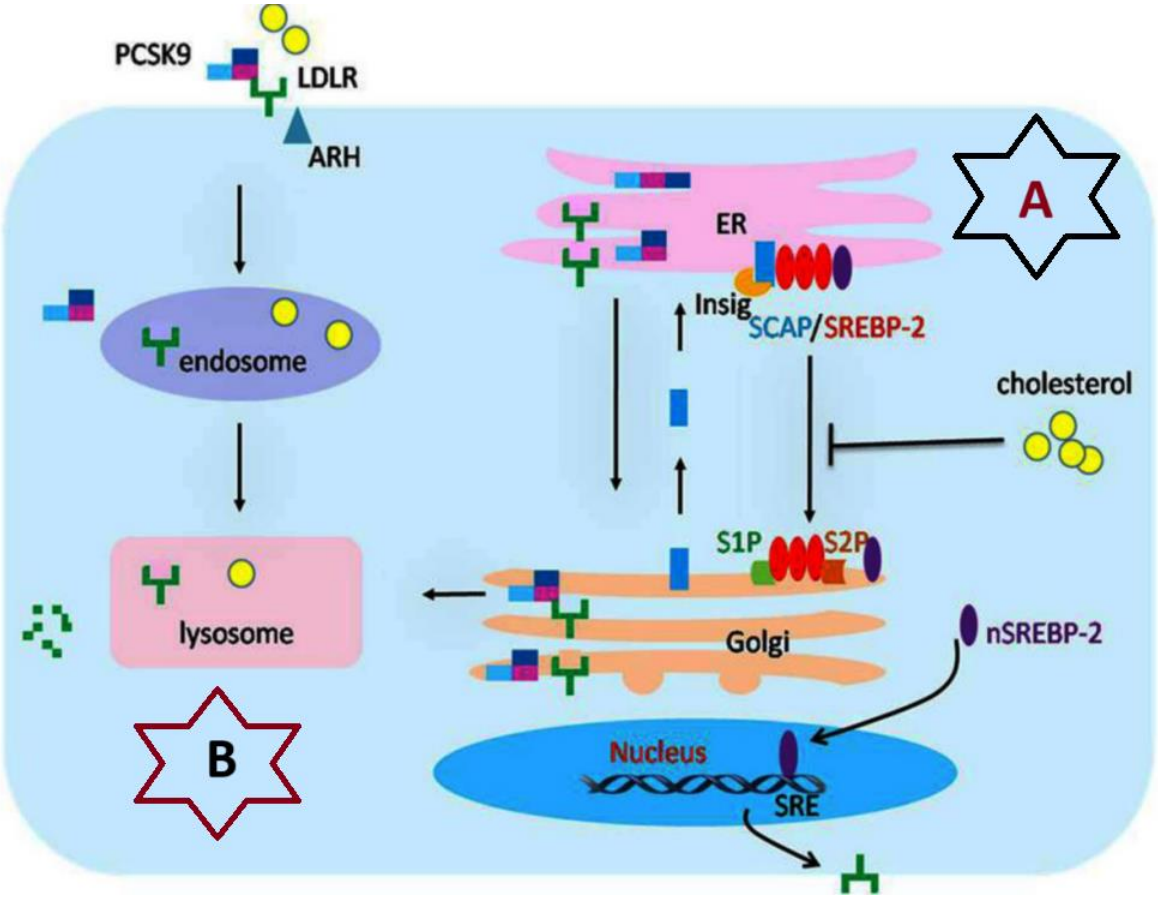


Figure (3):Transcriptional and post-transcriptional regulation of LDLR expression, adapted from *(Zhang, Ma, Ruan, & Liu, 2016)*. Part (A): when cholesterol is high inside the cell, SCAP undergoes a conformational change and interacts with Insig to form SCAP-SREBP-2-Insig complex, and SREBP-2 is trapped in ER, so LDLR expression is inhibited. When cell cholesterol is low, SCAP does not interact with Insig, so SCAP-SREBP-2 is free to leave the ER, then SREBP-2 gives the mature form, which enters the nucleus, binds to SRE and promotes LDLR expression. Part (B): when the cell does not need extra cholesterol, PCSK9 is secreted and binds specifically to LDLR. After binding LDL-C and being internalized to the cell, PCSK9 prevents LDLR conformational changes, which release LDL-C, and instead, still bound to the complex and transported to the lysosome where LDLR is degraded with LDL-C and PCSK9. ER: Endoplasmic Reticulum; LDLR: Low Density Lipoprotein Receptor; PCSK9: Proprotein Convertase Subtilisin Kexin 9; SREBP-2: Sterol Regulatory Element Binding Protein 2; SRE: Sterol Regulatory Element; nSREBP-2: Nuclear SREBP-2; SCAP: SREBP Cleavage Activating Protein.

Pathogenic variants in the *LDLR* gene are the most common cause of FH and contribute to 85-90% of genetically confirmed FH cases. More than 2600 unique variants in the LDLR gene were submitted to the ClinVar database. Most of them were missense mutations followed by frameshift

mutations, and around 18% of all unique variants were located in exon 4 (Iacocca et al., 2018). Pathogenic variants in the *LDLR* gene can impair LDLR activity at different levels. Class 1 mutations result in no protein synthesis (null variant), class 2 give partial or complete retention of LDLR in the ER, class 3 contribute to defective binding to apolipoprotein B (apo B), class 4 results in an impaired endocytosis and class 5 raise diminished LDLR recycling capacity. The amount of plasma LDL-C is inversely linked to LDLR activity. As a result, patients with null variants have the most severe phenotype (highest LDL-C), whereas receptor-deficient patients have lower LDL-C levels dependent on LDLR functional reserve (Galicia-Garcia et al., 2020).

Of the five LDLR domains, the EGF Precursor Homology Domain has the highest frequency of missense mutations all over the LDLR gene, with around 1108 missense variants localized through exons 7 to 14 representing this domain. It consists of two EGF-like domains (EGF-A and EGF-B), six YWTD repeats forming a six-bladed β-propeller, and a third EGF-like repeat (EGF-C). Any variant will strongly be FH-causing because this domain is essential in lipoprotein release and receptor recycling. Figure (4) shows the location and frequency of variants in this domain (Galicia-Garcia et al., 2020).

2.2.3.2 APOB gene

The Apolipoprotein B (*ApoB*) gene is located on the short arm of chromosome 2 (2p24.1) and has 29 exons. This gene extends over 43 kilobases. There are two different isoforms of apoB found in the plasma: apoB100 and apoB48. ApoB100 is the larger one, produced in the liver, composed of 27 amino acid signal peptides followed by 4536 amino acids forming the mature protein, and it plays a role in LDL-C packing. Meanwhile, apoB48 is produced in the intestine and formed by a stop codon generated at residue 2153 by RNA editing. It also plays a role in chylomicron packing (Blackhart et al., 1986).

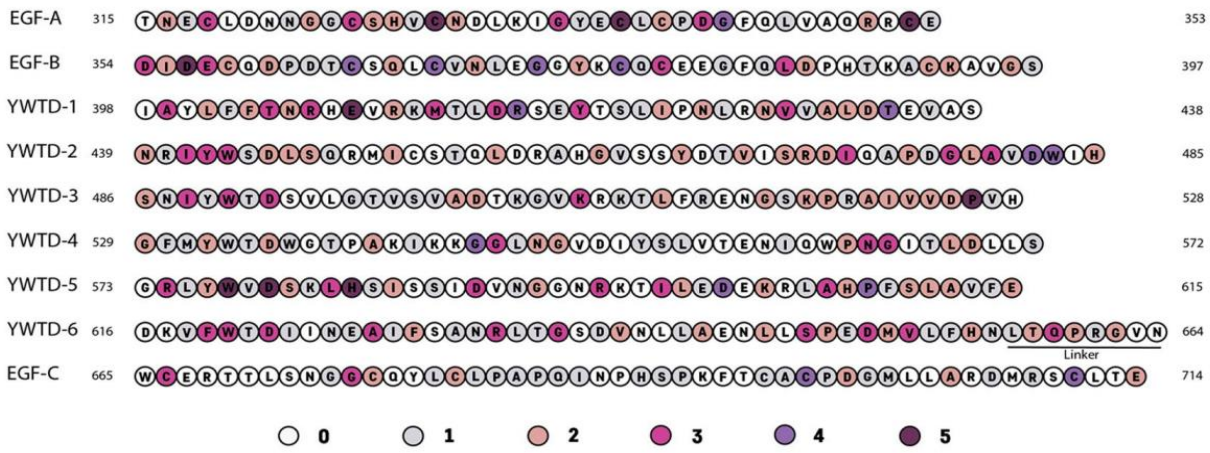


Figure (4): Frequency and location of LDLR variants within the EGF Precursor Homology Domain according to ClinVar database, adapted from *(Galicia-Garcia et al., 2020)*. Colors indicate the number of described variations for a specific amino acid.

ApoB100 is highly insoluble in water and is the only LDL-C protein component. ApoB100 is one of the largest monomeric proteins known. It has at least 25% amphipathic α-helices in its structure and has five domains containing trypsin releasable and non-releasable regions rapping the LDL-C particle, as shown in Figure (5) (Orringer & Grant, 2020).

ApoB100 is essential for the assembly of VLDL-C in the liver and acts as the primary ligand for LDLR, mainly on hepatic cell surfaces. Any variant in the gene responsible for its production could be pathological and FH-causing. Missense variants are the most common, followed by synonymous variants. 41% of these variants were found in exon 26 and 15% in exon 29 of the *APOB* gene (Iacocca et al., 2018).

Five identified mutations in the *ApoB-100* gene confirm FH with *APOB.* Each mutation affects a single protein building block in ApoB-100 crucial areas. The Val2095Glu variant has a greater risk of developing FH in any population. A case-control study held in Saudi Arabia and published in 2019 found that there is an association between the rs151009667 polymorphism (c.5066G>A, p. Arg1689His) and FH in a Saudi population, but Val2095Glu appeared neither in patients nor controls (Batais et al., 2019). Arg3527Gln (R3527Q) variant can be found in Caucasian patients with FH. The mutation is also widely prevalent in Central Europe (Germany, Switzerland and Belgium). The Arg3527Gln mutation was recently found in Bulgaria, but it was absent from

patients in Turkey. A case report study confirmed the presence of Arg3527Gln variant in Greece in 2013(Markoula, 2013).

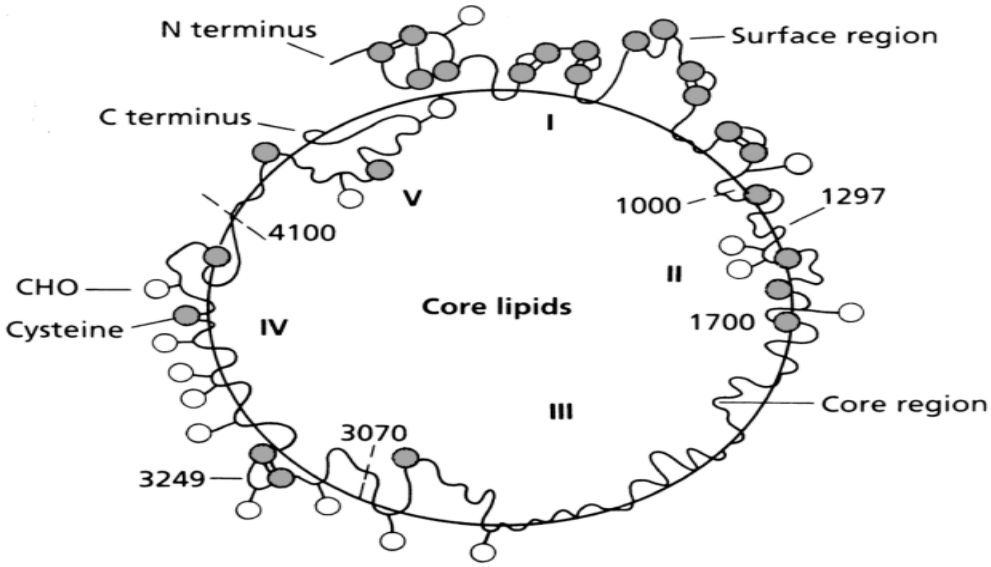


Figure (5*)*: Schematic diagram of the structure of apoB-100 on the surface of LDL-C, adapted from *(Schumaker, Phillips, & Chatterton, 1994)*. The trypsin-releasable and trypsin-nonreleasable areas are depicted inside and outside the LDL-C surface, respectively. The two thrombin-cleavable sites are identified at residues 1,297 and 3,249. The five suggested domains are separated by dashed lines. The open circles indicate N-glycosylated carbohydrates, whereas the darkened circles represent cysteine residues.

2.2.3.3 PCSK9 gene

The Proprotein Convertase Subtilisin/Kexin 9 (*PCSK9)* gene is located on chromosome 1short arm (1p32.3) and comprises 15 exons. PCSK9 gene encodes the 9th member of the subtilisin-like proprotein convertase family. This family contains proteases that process protein and peptide precursors that go through controlled or constitutive branches of the secretory route. PCSK9 was first described in 2003 by Nabil Seidah and Jae Byun. The produced protein contains 692 residues that are divided into signal peptide (residues 1-30), the N-terminal prodomain (residues 31-152), the catalytic domain (residues 153-425), and the C-terminal domain (residues 426-692) (Zhang, Ma, Ruan, & Liu, 2016).

Several studies showed that PCSK9 works as a posttranscriptional regulator of LDLR expression. Figure (6) explains the role of PCSK9 in LDLR expression. PCSK9 catalytic domain binds to the EGF Precursor Homology Domain A of LDLR prevents the dissociation of LDLR and LDL-C. Then, the whole complex is sent to the lysosome and degraded there. This decreases the available LDLR on the cell surface, thus increasing serum LDL-C levels (Lin et al., 2018). At the transcriptional level, PCSK9 expression is regulated by SREBP-1 and SREBP-2, which bind SRE in PCSK9 promotor and therefore upregulate PCSK9. Upregulation of PCSK9 typically occurs when the LDL-C level is low, so PCSK9 decreases available LDLR and LDL-C internalization to the cell. PCSK9 promotor also contains a binding site for hepatocyte nuclear factor 1 alpha (HNF1α), and silencing HNF1α reduces PCSK9 expression significantly (Xia et al., 2021).

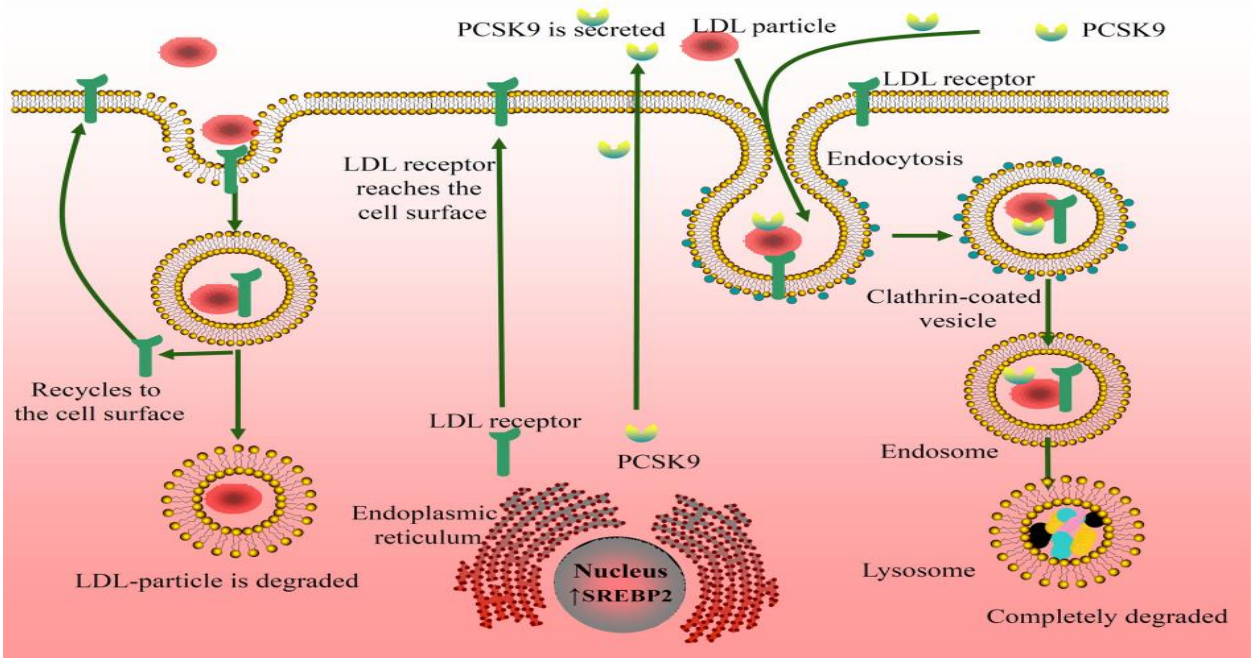


Figure (6): Schematic representation of the intracellular and extracellular pathways of PCSK9-induced degradation of the LDLR, adapted from *(Lin, Xiao, Tang, Jiang, & Liu, 2018)*. Elevated PCSK9 levels promote LDLR breakdown in acidic lysosomes, which raises LDL-C levels in the blood. When PCSK9 is absent, LDLR remains on the cell surface, where it carries LDL-C particles to acidic endosomes for hydrolysis before being recycled back to the surface.

In 2004, the first gain of function (GOF) variants at the PCSK9 gene were reported. Timms et al. (2004) identified a single G→T nucleotide variation on the K1173 haplotype, resulting in the non-synonymous p.(D374Y). Leren discovered an asparagine to lysine substitution at position 157, p. (N157K). Benjannet et al. (2004) found two more naturally occurring PCSK9 variants, p.(R218S) and p.(R237W). Shioji et al. (2004) found a link between total cholesterol (TC) and LDL-C levels with exon 9/I474V or intron 1/C (-161) T polymorphisms (Guo, Feng, & Zhou, 2020). Di Taranto et al. (2017) reported two variants (p. (Ser636Arg) and p. (Arg357Cys)), and they were GOF variants (Di Taranto et al., 2017). Table (1) demonstrates the most common GOF variants among different populations.

Table 1: Summary of GOF variants in the PCSK9 gene among different populations, adapted from *(Guo et al., 2020).*

Researchers	*PCSK9* variants	Populations	Sample size	Variant frequency, %	LDL-C ranges, mmol/L
Leren (2004)	D374Y	Norwegian, FH	51	5.9	7.0–10.6
Allard et al. (2005)	R357H	French, FH	130	0.8	4.3–6.2
	R469W	French, FH	130	0.8	6.0–9.2
Humphries et al. (2006)	D374Y	British, FH	409	1.7	1.82–6.77
Taylor et al. (2007)	D374Y	British, FH	400	2.2	>4.9
Bourbon et al. (2008)	D374H	Portuguese, FH and relatives	602	0.5	4.9–9.4
Noguchi et al. (2010)	E32K	Japanese, FH	55	6.4	5.8–8.8
Abifadel et al. (2012)	D35Y	French, ADH	75	2.7	6.0
Mabuchi et al. (2014)	E32K	Japanese, FH	1,055	5.9	8.0–16.0
Ohta et al. (2016)	V4I	Japanese, FH	269	6.3	4.5–7.8
	E32K	Japanese, FH	269	6.3	4.5–7.8
	R496W	Japanese, FH	269	0.4	4.5–7.8
Xiang et al. (2017)	R96L	Chinese, FH	219	0.5	4.5–12.2
	R105W	Chinese, FH	219	0.5	4.5–12.2
Kaya et al. (2017)	D374Y	Turkish, FH	80	5.0	2.2–6.5
	R496W	Turkish, FH	80	8.7	2.0–9.8
Eroğlu et al. (2018)	D374Y	Turkish, dyslipidemia	200	7.0	3.7–8.7
	R496W	Turkish, dyslipidemia	200	6.5	3.7–8.7
Luirink et al. (2019)	A220T	Netherlander, FH	1,903	0.1	7.7–9.0

Normal range of LDL-C: Low-risk groups < 3mmol/L; High-risk groups < 1.8 mmol/L; FH groups < 1.4 mmol/L. (According to the 2019 ESC/EAS guidelines for the management of dyslipidaemias). PCSK9, proprotein convertase subtilisin/kexin type 9; LDL-C, low-density lipoprotein cholesterol; FH, familial hypercholesterolemia; ADH, autosomal dominant hypercholesterolemia.

Loss of function (LOF) mutations are believed to be FH-protecting variants. In a study of 128 individuals with low plasma LDL-C levels, Cohen et al. (2005) discovered two nonsense variants, p.(C679X) and p.(Y142X), by analyzing the PCSK9 coding sequence. Since 2005, these PCSK9 variations have been classified as LOF due to their counter impacts in contrast to the GOF variants.

Table (2) summarizes LOF variants among different populations (Guo et al., 2020). Furthermore, Berge et al. (2005) found four missense LOF variants that cause hypocholesterolemia and may enhance the effect of statin therapy (Berge, Ose, & Leren, 2006).

Table 2: Summery of LOF variants in PCSK9 gene among different populations, adapted from *(Guo et al., 2020).*

Researchers	*PCSK9* variants	Population	Sample size	Variant frequency, %	LDL-C ranges, mmol/L
Cohen et al. (2006)	Y142X	ARIC study, general	3,363	0.8	1.7–3.7
	C679X	ARIC study, general	3,363	1.8	1.4–3.8
	R46L	ARIC study, general	9,524	3.2	2.2–3.9
Hooper et al. (2007)	C679X	African, general	653	3.7	1.3–1.9
Scartezini et al. (2007)	R46L	British, general	2,444	1.0*	1.8–4.0
Guella et al. (2010)	R46L	Italian, MI patients	1,880	1.0*	2.1–3.9
Chernogubova et al. (2012)	R46L	Swedish, general	5,722	1.9*	2.5–4.9
Saavedra et al. (2014)	R46L	Canadian, FH	582	3.0	5.8–7.7
Langsted et al. (2016)	R46L	CGPS study	103,083	1.3*	2.2–3.4
Mostaza et al. (2018)	R46L	Spanish, adults	1,188	2.9*	2.6–4.3
	R46L	Spanish, children and adolescents	1,933	3.2*	2.2–2.6

*Normal range of LDL-C: Low-risk groups < 3 mmol/L; High-risk groups < 1.8 mmol/L; FH groups < 1.4 mmol/L. (According to the 2019 ESC/EAS guidelines for the management of dyslipidaemias). PCSK9, proprotein convertase subtilisin/kexin type 9; LDL-C, low-density lipoprotein cholesterol; ARIC, Atherosclerosis Risk in Communities; MI, myocardial infarction; CGPS, Copenhagen General Population. *Represents the allele frequency which is different from the PCSK9 variant proportion of the population in the different references.*

2.2.4 Diagnosis of FH

Recent studies have shown that FH diagnosis and treatment are significantly below the wanted range. FH registry showed that FH diagnosis usually took place after the patient had experienced an ASCVD event by a mean age of 50 (McGowan et al., 2019).

Routine lipid screening in children between 9 and 11 years before puberty, as the American Pediatric Association, American Heart Association and American College of Cardiology recommends, can improve case findings (McGowan et al., 2019). Slovenia implemented the first universal pediatric FH screening scheme in 1995. The program employed a two-step approach: universal hypercholesterolemia screening (1st step) in pre-school children (5 years old) during their scheduled visit to the primary care paediatrician, where total cholesterol was measured, and genetic FH testing (2nd step) in children with elevated total cholesterol (> 90mg/dL). These

children were then moved to tertiary care (lipid clinic). (Medeiros & Bourbon, 2023). Early screening of lipid status at the age of two years is advised if the kid has a significant family history of premature CAD, a parent with a total cholesterol level >240 mg/dL, or if the kid has underlying cardiovascular risk factors such as obesity or diabetes (McGowan et al., 2019).

2.2.4.1 Clinical diagnosis of FH

Diagnosis of FH is used clinically using clinical criteria to classify patients into categories describing the probability of having FH. It is based on a combination of physical symptoms, personal or family history of hypercholesterolemia and early coronary artery disease, and plasma LDL-C levels (McGowan et al., 2019).

Physical findings vary between individuals. As LDL-C accumulates in arterial walls, it accumulates in tendons presented as xanthomas, in the cornea, which gives rise to corneal arcus and around the eyes as xanthelasmata; see figure (7) (Schmidt et al., 2020).

Xanthomas are formed by collagen and foam cells deposition on tendons, causing local thickening of fascia, tendons and ligaments. They are frequently found on extensor surfaces of elbows, knees, knuckles and hands, Achilles tendon and buttocks. Although this physical finding could be pathognomonic to FH, it could also be seen in sitosterolemia, cerebro-tendinous xanthomatosis and familial dysbetalipoproteinemia (type III dyslipidemia) (Schmidt et al., 2020).

Corneal deposition of LDL-C results in corneal arcus, and it is generally found in older adults, but in the case of FH, this process is accelerated and pathologically found in young individuals. Corneal arcus is asymptomatic and does not affect vison accuracy or eye function (Schmidt et al., 2020).

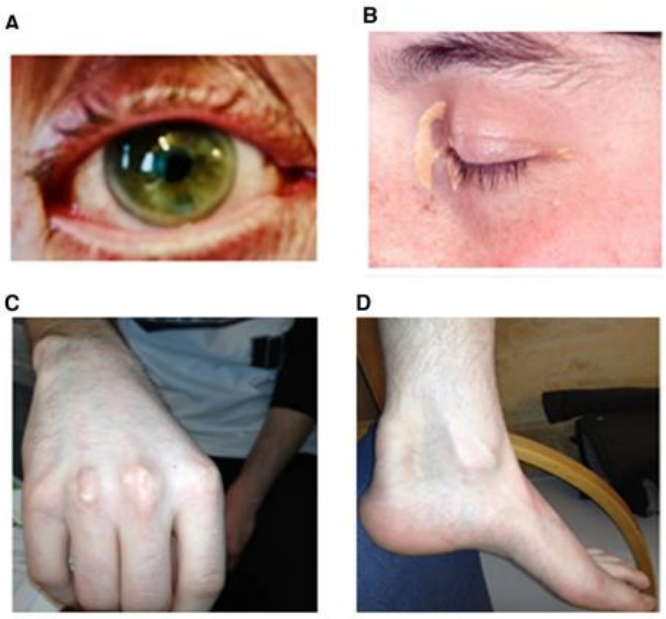


Figure (7): Physical finding of familial hypercholesterolemia, adapted from *(Schmidt et al., 2020).* A: Corneal arcus. B: Xanthelasma. C. Tendon xanthoma. D: Achilles tendon xanthoma.

Xanthelasmata are yellowish plaques around the eye, and they are not specific for FH and may be found in people with an average cholesterol level. An interesting finding in a recent study from Copenhagen showed that individuals who have xanthelasmata are at increasing risk of having CVD, whatever the cholesterol level is (Schmidt et al., 2020). Fasting LDL-C is a part of most clinical diagnostic criteria, while non-fasting lipid measures can be used for initial screening. High LDL-C above the 95th percentile (>190mg/dL) is central for FH clinical diagnosis (Schmidt et al., 2020). Putting all together, FH clinical diagnosis is suspected when the patient has elevated LDL-C, a personal or family history of premature CAD and a family history of hypercholesterolemia (Schmidt et al., 2020).

As mentioned, many systems are used as clinical diagnostic criteria for FH. Our review focuses on two of them: the UK Simone Broome Criteria and the Dutch Lipid Clinical Network Criteria (DLCN). Simone Broome's criteria consider cholesterol plasma level, physical findings, and family history. The criteria are summarized in Table (3). **Advantages** of these criteria are: (1) the criteria were laid out in a manner to be easily recalled, making their application simple in daily clinic use; (2) Simone Broome criteria at a glance, employ traditional physical findings, history taking and non-invasive lipid profile study to categorize patients to have possible or definite FH, which implements this criterion simple and cost-effective. **Disadvantages** of Simone Broome Criteria are: (1) it failed to discriminate between mutations causing FH such as LDLR, ApoB100

or PCSK9 genes mutations, (2) and it will not differentiate between FH and non-FH hypercholesterolemia. From here, the need for an update to Simone Broome criteria emerged, and DLCN criteria were carried out (Luirink et al., 2019).

DLCN criteria were the modification of Simone Broome's criteria. DLCN criteria depend on the scoring system divided into personal or family history of premature CAD and family history of hypercholesterolemia that is inherited in an autosomal manner, LDL-C plasma level and DNA analysis of functional mutation of LDLR, ApoB100 or PCSK9 genes. Then, subjects will be categorized as definitely, probably, possibly or unlikely to have FH according to their scores. DLCN criteria are summarized in Table (4). The main **advantage** of DLCN is that it focuses on the molecular defect causing FH, which will help in the management plan. **Disadvantages** of DLCN are: (1) it lacks flexibility to be used in daily clinics, especially if there is a large number of patients attending that clinic, as calculating the score for each patient could be time-consuming; (2) it is considered expensive since DNA analysis is a part of the scoring system (Luirink et al., 2019).

Table 3: Simone Broome Criteria for FH clinical diagnosis, adapted from *(McGowan et al., 2019).*

Criteria	Possibility
In adults: TC >7.5 mmol/L (290.0 mg/dL) (or when available, LDL-C >4.9 mmol/L [189.5 mg.dL]) In pediatric patients: TC >6.7 mmol/L (259.1 mg/dL), or LDL-C >4 mmol/L (154.7 mg/dL), AND	Definite
Tendon xanthoma in the patient or first/second-degree relative, OR alternatively:	
Presence of LDL-R, ApoB, or PCSK9 mutation	
In adults: TC >7.5 mmol/L (290.0 mg/dL) (or when available, LDL-C >4.9 mmol/L [189.5 mg.dL]) In pediatric patients: TC >6.7 mmol/L (259.1 mg/dL), or LDL-C >4 mmol/L (154.7 mg/dL), AND	Possible
Family history of MI <50 y old in second-degree relative or <60 y old in first-degree relative OR alternatively	
Family history of TC >7.5 mmol/L (290.0 mg/dL) in a first- or second-degree relative.	

By comparing these criteria, if the physician looks for accuracy in diagnosing FH and the clinic is not crowded, DLCN is better to use. Simone Broome criteria is better to use during flexibility and ease of use for many patients attending the clinic (Luirink et al., 2019).

2.2.4.2 Genetic diagnosis of FH

Although clinical diagnosis of FH can be reliable most of the time, clinical criteria may miss around half the cases. On the other hand, about 4-8% of patients fulfilling the clinical criteria were found to have negative genetic testing (Cao et al., 2018). Also, genetic testing of FH offers a definite molecular diagnosis defining the exact gene mutation causing the disease, an index for cascade screening, prognosis for each patient, and CVD risk stratification for those patients with FH. It addresses the appropriate management plan for them (Medeiros & Bourbon, 2023).

Over the years, advances have been made in FH genetic testing, reducing costs. The most effective and easy method used nowadays is the expanded-FH Next Generation Sequencing (NGS) panel, which allows the study of FH-causing genes, LDLR, PCSK9, ApoB100, in addition to other relevant genes like APOE, LIPA, and ABCG5/8 (FH phenocopy genes) (Medeiros & Bourbon, 2023).

Whole genome molecular study is consuming time due to the high number of probable FH patients. A novel genotyping DNA microarray chip, an FH chip based on arrayed primer extension (APEX), offers a speed up in variant catching in Czech FH patients. This study demonstrated that the validation phase of the FH chip has 100% sensitivity and 99.1% specificity. Researchers proposed using the FH chip for quick, reproducible, specific, and cost-effective genotyping. According to a study conducted in 2016, NGS costs five times more than analyzing and reporting a single sample utilizing FH Biochip technology, which offers greater sensitivity and quicker detection (Guo et al., 2020).

2.2.5 Cascade screening

In 1997, the World Health Organization recognized the benefits of genetic diagnosis of FH, but they assumed it was expensive to screen a population with this type of testing. A new concept emerged to solve this issue: the cascade screening. It depends on diagnosing a patient with FH using the available clinical criteria, then confirming the diagnosis by genetic testing and addressing the genetic variant causing FH. After that, screening among first-degree relatives for matching

mutations is applied. New cases of affected first-degree relatives are treated as a new index, and their first-degree relatives are screened. This concept made the diagnosis of FH among a homogenous population easy and cost-effective. It was used in the Dutch, Spanish, Norwegian, Australian, Wales and New Zealand populations. This strategy can be used in homogenous Arab societies (Al-Rasadi et al., 2014).

Table 4: Dutch Lipid Clinic Network (DLCN) criteria for FH clinical diagnosis, adapted from *(McGowan et al., 2019).*

Criteria	Score
Family history	
Premature CVD (men <55 y old, women <60 y old) in first-degree relative, OR	1
LDL >95th percentile in first-degree relative AND/OR	1
Tendon xanthoma and/or arcus cornealis in first-degree relative, OR	2
LDL >95th percentile in children <18 y old	2
Personal history	
Premature CAD in patient (men <55 y old, women <60 y old)	2
Premature cerebral or peripheral vascular disease (men <55 y old, women <60 y old)	1
Clinical examination	
Tendon xanthomas, OR	6
Corneal arcus younger than 45 y old	4
LDL	
>330 mg/dL (8.5 mmol/L)	8
250–329 mg/dL (6.5–8.5 mmol/L)	5
190–249 mg/dL (4.9–6.4 mmol/L)	3
155–189 mg/dL (4.0–4.9 mmol/L)	1
Presence of functional LDL-R mutation (in the LDL-R, ApoB, or PCSK9 gene)	8
Diagnosis based on the overall score	
Definite	>8
Probable	6–8
Possible	3–5
Unlikely	<3

2.2.6 Management of FH

Dietary and lifestyle changes are first recommended to decrease LDL-C levels in FH patients. However, multidrug strategy may be necessary to attain sufficient LDL-C levels (McGowan et al., 2019). 2021 European Society of Cardiology (ESC)guidelines stated that the LDL-C target in HeFH without ASCVD or significant risk factors was lowered from <100 mg/dL to <70 mg/dL,

and with ASCVD or significant risk factor was lowered from <70 mg/dL to 55mg/dL. While the treatment goal for children aged ten years and older remained unchanged at <130 mg/dL, as suggested by the American Academy of Pediatrics in 1992 (Langslet, Holven, & Bogsrud, 2022). Several studies approved that the early introduction of statins reduces the production of coronary events. One observational cohort study followed FH patients taking statin therapy for 8.5 years and found that statin therapy reduces cardiovascular events by 76%. Besides pharmacological treatment, patients with FH should be advised to avoid smoking and vaping, engage in regular exercise, and maintain a healthy weight. ASCVD risk factors and comorbidities should be treated and controlled, including hypertension and diabetes (McGowan et al., 2019).

Statins, ezetimibe, bile acid sequesters, and PCSK9 inhibitors are the drugs that are available to treat dyslipidemia, especially FH. Statins are HMG-CoA reductase inhibitors that block the first step of cholesterol synthesis. Also, statins were found to increase LDLR expression, thus indirectly decreasing circulating LDL-C levels. Statin use is divided into three strategies: high-intensity, moderate-intensity and low-intensity, as shown in figure (8). Ezetimibe targets NPC1L1 to decrease cholesterol absorption from the intestines. The concurrent treatment of high-intensity statin with ezetimibe in patients with acute coronary syndrome was demonstrated to strongly regulate LDL-C levels and reduce future cardiovascular mortality, nonfatal MI, unstable angina necessitating hospital admission, coronary revascularization, and nonfatal stroke when compared to statin alone use (McGowan et al., 2019).

If LDL-C level is not sufficiently controlled, bile acid sequestrants are an excellent choice to add on statin and ezetimibe. Those agents, like colesevelam, bind to bile acids in the intestines and form an insoluble complex that is eliminated in faeces and blocks the action of bile acid to emulsify lipids, preparing them for absorption. Colesevelam can provide 14-18% additional LDL-C level reduction when added to statin and ezetimibe. Other agents like cholestyramine and colestipol can substantially reduce LDL-C when used in higher-tolerated doses (McGowan et al., 2019).

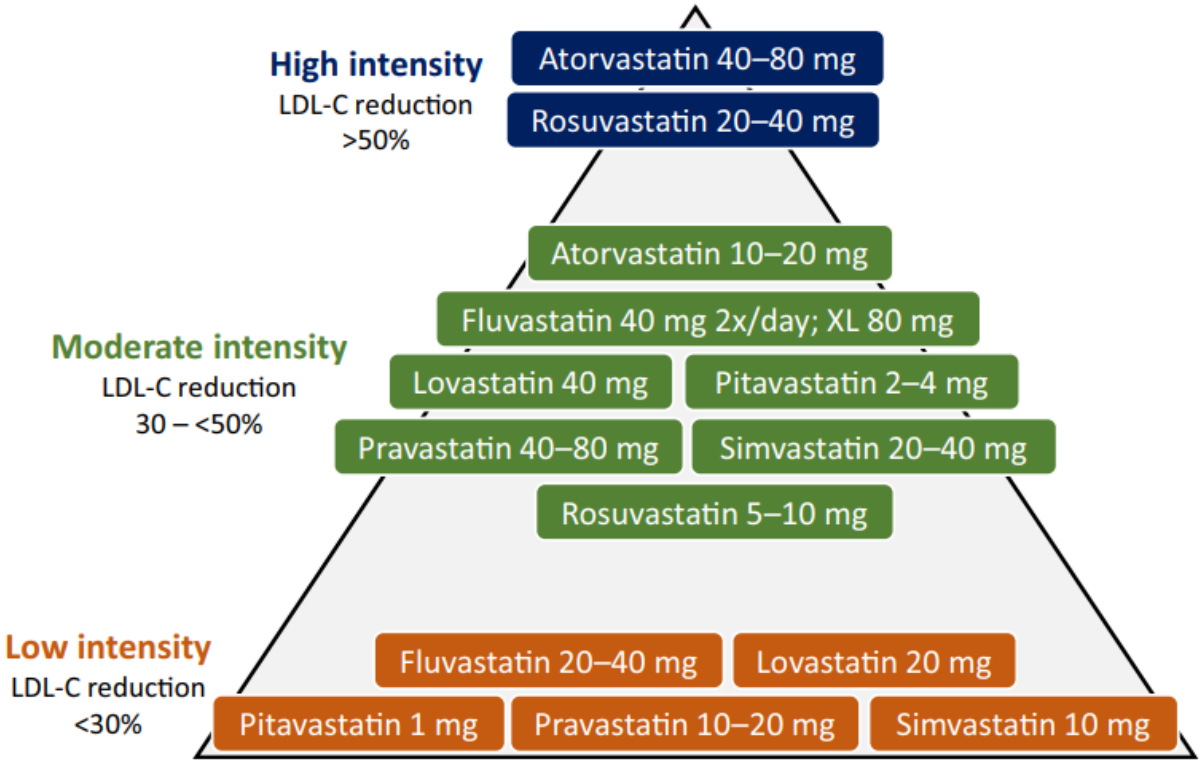


Figure (8): Statin use strategies, adapted from *(McGowan et al., 2019)*

Available PCSK9 inhibitors (alirocumab and evolocumab) are human monoclonal antibodies that target PCSK9 and increase available LDLR, thus decreasing circulating LDL-C as monotherapy or add-on therapy to statin and ezetimibe (McGowan et al., 2019).

ODYSSEY I and ODYSSEY II studies revealed that alirocumab was well-tolerated and significantly reduced LDL-C levels in individuals with HeFH who had insufficient control at baseline, even with statins and other LLTs (Kastelein et al., 2015). Among FH patients who are intolerable for statin due to myalgia, PCSK9 monoclonal antibodies (evolocumab) provide an added benefit over the use of ezetimibe in reaching the target LDL-C level (Nissen et al., 2016).

If LDL-C level remains > 100mg/dL after maximal tolerable multidrug therapy for FH patients, lipoprotein apheresis could be used to wash out LDL-C from circulation using the Dextran Sulfate Low Density Lipoprotein Adsorption system (McGowan et al., 2019).

2.2.6.1 Therapies for pediatric patients

Lifestyle modification is the first step in management for diagnosed FH patients from children and adolescents. Suppose 3-6 months passed without reaching the LDL-C goal of <130 mg/dL if >10 years or 50% reduction from baseline if 8 to 10 years of age. According to several guidelines, statins should be added to patients aged 8 years or older. The only approved statins for 8-year-old children are simvastatin and pravastatin, while the remaining statins can be added for ten years and older (McGowan et al., 2019). A study was published in 2019 for a 20-year follow-up of using statin therapy in 214 FH children in Amsterdam, Netherlands. It showed that starting statin therapy for patients with FH from early childhood decreases the carotid intimal thickness (a parameter used to evaluate atherosclerosis) and minimizes CVD risk during adulthood (Luirink et al., 2019).

According to several studies, Ezetimibe can be added to reach LDL-C targets in patients older than ten. A previous prospective, multi-centre, placebo-controlled study found that the addition of ezetimibe to simvastatin in treating patients with HeFH older than ten years provided 16% more reduction in LDL-C level when compared with using simvastatin alone without noticeable additional side effects. Also, colesevelam can be used alone or as add-on therapy for boys older than ten years and postmenarchal girls, and it reduces LDL-C by 13-18%. On the other hand, PCSK9 inhibitors have not been studied adequately for use in children with FH (McGowan et al., 2019).

2.2.6.2 The concept of primary prevention

Primary prevention refers to the actions made by an individual to avoid a particular disease. This is accomplished by following a healthy lifestyle, such as food and exercise. Also, the risk factors for that disease should be screened and managed. Secondary prevention aims to reduce the disease's effect by early detection before it causes severe and lasting harm. This helps to avoid life-threatening circumstances and long-term disease-related disabilities (Thongtang, Sukmawan, Llanes, & Lee, 2022).

In the case of dyslipidemias, including FH, an asymptomatic course is a dangerous fact about it. Screening is the cornerstone of managing this silent disease to prevent its more severe complication, ASCVD. Primary prevention is indicated to prevent or slow the progression of ASCVD before the patient has a cardiovascular event. The best approach is access to primary preventive tools including screening, risk estimation, diagnosis, and management with statin or non-statin drugs (Thongtang et al., 2022).

2.2.6.3 Gene therapy

Li et al. proposed an exosomal prodrug for the first time by encapsulating therapeutic *LDLR*. They engineered an exosome, injected it with a plasmid that carries the wildtype of *LDLR* and treated *LDLR*-null mice after eight weeks of a lipid-rich diet. They found that LDLR expression increased among those mice, and fatty liver characteristics decreased. They believe it is a promising therapeutic tool, especially for FH patients without lipid-lowering agents. Figure (9) represents the technique they used in their experiment. They explained using exosome instead of viral vector by immune response, as viral vector exacerbates an immune response while exosome can be structured based on individual-specific cells to prevent any immune response. On the other hand, they did not use liposomes as macrophages rapidly cleared them (Li et al., 2021).

It had been reviewed that a trial of targeting PCSK9 was introduced using CRISPR/CRISPR-associated (Cas) technology to acquire a PCSK9-targeting CRISPR guide RNA and Cas 9 expression in murine livers, resulting in reducing PCSK9 levels, boosting available LDLR on the cell surface, and lowering plasma LDL-C levels (Guo et al., 2020).

2.2.7 Complications of FH

2.2.7.1 Risk of ASCVD

Atherosclerosis is the formation of intimal lesions, defined as atheromas, that narrow the vascular lumen and may rupture to produce a clot and cause sudden vascular occlusion. Atherosclerosis is the underlying cause of coronary, cerebral and peripheral vascular disease. Those atheromas are

lipid cores containing cholesterol, cholesterol esters and necrotic debris encountered by a fibrous cap (Kumar, 2018).

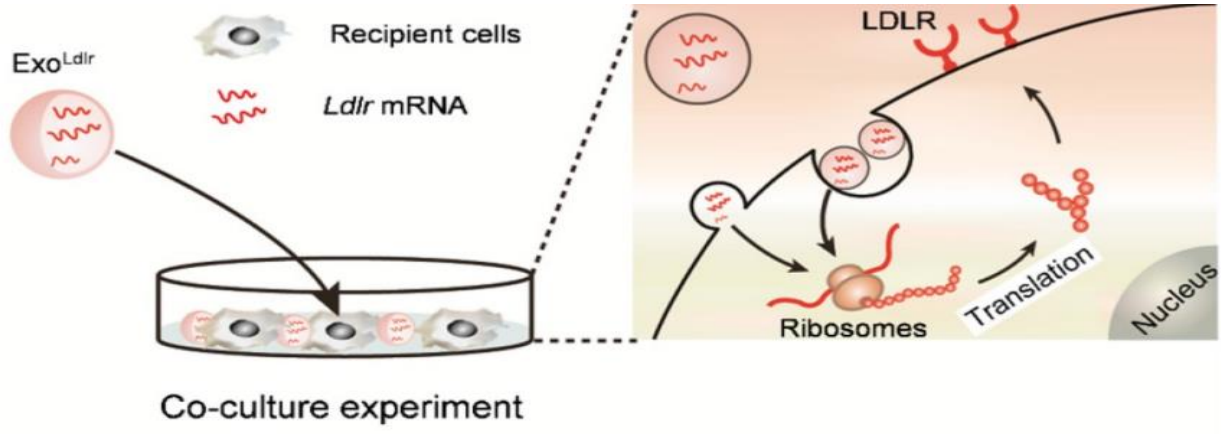


Figure (9): Schematic illustration of the exosome-mediated LDLR mRNA delivery into the recipient cells, where the mRNA is translated into the functional protein, adapted from *(Li et al., 2021).*

The main risk factors for atherosclerosis are genetic causes, age, gender, dyslipidemia, hypertension, cigarette smoking and diabetes mellitus. Dyslipidemia, specifically hypercholesterolemia, is a major risk factor and is sufficient to cause atheroma formation without coexisting with other risk factors (Kumar, 2018).

The pathogenesis of atherosclerosis is built on the response-to-injury hypothesis, which means that atheromas are formed in response to chronic inflammatory processes resulting from endothelial injury. LDL-C particles became oxidized by reactive oxygen species (ROS), and oxidised LDL-C is thought to be highly atherogenic and thrombogenic. Once high levels of LDL-C are circulating in the blood, a high concentration of oxidized-LDL-C will be formed and deposited to the endothelium (Batty, Bennett, & Yu, 2022).

Oxidized-LDL-C with other atherosclerosis risk factors such as smoking, hypertension and formation of advanced glycated end-products (AGEs) in diabetes mellitus, together, stimulate endothelial dysfunction. Dysfunction urges endothelial cells to express adhesion molecules and chemokines that cause the recruitment of monocytes and T-lymphocytes. Monocytes then

transmigrate to subintimal space to differentiate into pro-atherogenic macrophages that harvest residual oxidized-LDL-C through their scavenger receptors and form lipid-rich foam cells. Those foam cells may undergo apoptosis and spill out their lipid contents. The spilt lipids and residual foam cells form the core of the atheroma or atherosclerotic plaque. In order to protect this plaque, vascular smooth muscle cells (VSMCs) are transformed into synthetic phenotypes and secrete extracellular matrix (ECM) components like collagen to form a fibrous cap that contains the plaque surface and prevents its rupture. When the plaque is ruptured due to necrosis, the thrombogenic lipid core is exposed, stimulating platelet aggregation and, thus, thrombus formation. This thrombus can move freely through the artery and may cause total or near-total occlusion, resulting in ischemia nad infarction of the downstream vascular territory (Batty et al., 2022); (Kumar, 2018). Figure (10) explains the development of atherosclerosis.

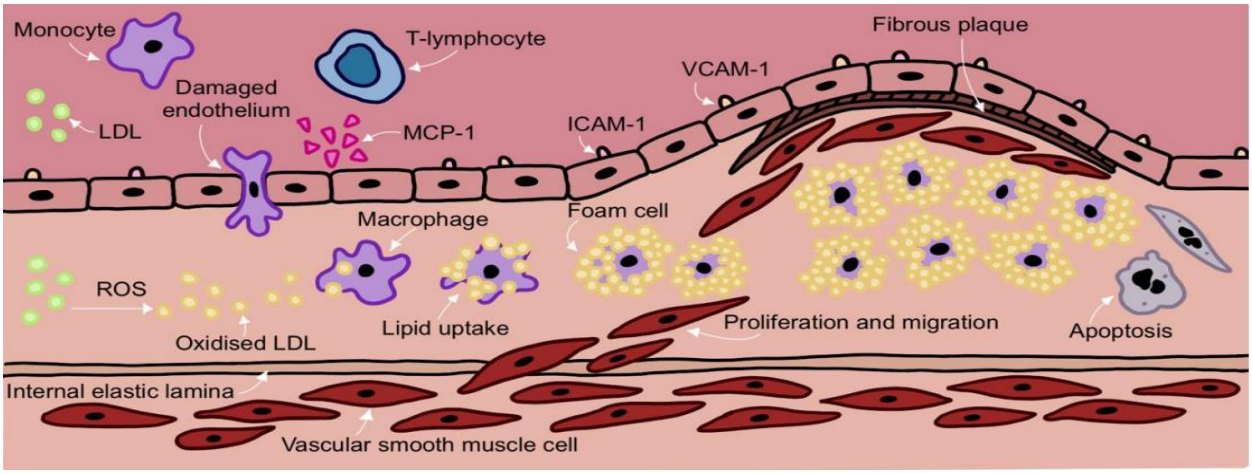


Figure (10): Overview of the development of atherosclerosis, adapted from *(Batty et al., 2022).* LDL-C is confined in the subendothelial region, where it is oxidized by ROS before being phagocytosed by macrophages. Foam cells are formed and then burst to spill out oxidized LDL-C particles, which are highly atherogenic. Accumulation of them promotes vascular smooth muscle cells and fibroblasts to form the fibrous cap. The more the accumulation, the larger the cap and the narrow the arterial lumen.

ASCVD development depends on two main factors: LDL-C level and exposure duration to high LDL-C. Since FH is a genetic disorder and elevated LDL-C is recognized early in life, it leads to 3-13 times more chance of developing premature ASCVD compared to the ordinary population. As patients with homozygous autosomal dominant FH have significantly higher levels of LDL-C

and more prolonged exposure since infancy, they are at a greater risk of developing ASCVD manifested by very premature CAD in their second decade of life (Poznyak, Litvinova, Poggio, Orekhov, & Melnichenko, 2022). Patients with monogenic FH and elevated LDL-C due to polygenic causes have a greater risk of developing ASCVD manifested by premature CAD, peripheral arterial disease (PAD), and chronic kidney disease (CKD) compared to normolipidemic individuals. However, at the same time, patients with monogenic FH have a greater risk and earlier presentation of premature CVD than patients with polygenic hypercholesterolemia or elevated LDL-C without genetic background due to the significantly higher levels of LDL-C and the longer time of arterial intima exposure to LDL-C since childhood (Emanuelsson, Nordestgaard, & Benn, 2018). Subsequently, FH patients with a single pathogenic gene mutation had a higher risk of mortality than those with polygenic hypercholesterolemia (McGowan et al., 2019). In a previous angiography study, 117 HeFH patients were screened for atherosclerotic lesions. The results showed that one-third had renal artery atherosclerosis, more than half had iliac atherosclerosis, 68% had abdominal aortic sclerosis, and 60% had CAD. The prevalence of PAD among FH patients was estimated to be between 1% and 31% and increases even more in HoFH. Also, several cross-sectional studies stated that FH increased the risk of having CKD due to renal artery stenosis and decreased eGFR with a prevalence of around 18% (Emanuelsson et al., 2018). On the other hand, no significant increase in the incidence of stroke was observed among FH patients compared to the ordinary population and extra investigations are warranted in this field (Svendsen et al., 2022).

2.2.7.2 Risk of aortic valve stenosis

It was reported that FH increases the risk of aortic valve calcification (AoVC), which may develop into aortic valve stenosis (AVS). The process starts with lipid infiltration, followed by inflammation, fibrosis and calcification. Management of hypercholesterolemia with either statins, ezetimibe or both would not strongly prevent the development of AVS as another contributor to the development of AoVC and AVS may exist, such as lipoprotein-a (Lp(a)) (Hu, Lei, Liu, & Xu, 2022). Lp(a) is an LDL-C-like particle containing apoB100 and plasminogen-like glycoprotein apo(a). High level of Lp(a) is a genetically inherited lipid metabolism disorder affecting 1.4 billion

around the world and may concomitantly exist with FH in at least 5 million subjects (Chubykina, Ezhov, Afanasieva, Klesareva, & Pokrovsky, 2022; Ellis et al., 2018). Since lipid-lowering medications work on lowering LDL-C, not Lp(a), it minimally prevents AVS development. An FH population's requirement for AVR owing to AVS was shown to be considerably more significant in the SAFEHEART long-term prospective cohort research. This was especially true for older patients, those with a history of ASCVD, hypertension, and long-term elevated plasma concentrations of LDL-C and Lp(a) (Hu et al., 2022).

2.2.7.3 CVD risk stratification

Refinement of risk stratification using measures of subclinical coronary atherosclerosis in asymptomatic FH people was found to be the most viable approach, even though several potential clinical and laboratory indicators might give additional prognostic information. It is essential to examine the role of atherosclerosis imaging in predicting the CVD risk in FH. The most studied imaging modalities in the FH population are carotid Intima/Media Thickness (cIMT), Coronary Artery Calcium score (CAC), and Coronary Computed Tomography Angiography (CCTA). CAC >0 is found in almost all FH patients with established ASCVD (96%), but non-calcified atherosclerotic plaques are not ruled out by CAC equal to 0 (Hu et al., 2022). Another way to evaluate ASCVD risk is to use the vascular age-Framingham risk score, in which values >20% are strongly associated with ASCVD development (Jeffrey Kolominsky et al., 2020).

2.2.8 FH in MENA region

Several studies from several regions worldwide reported specific FH-causing variants among specific populations. Here, we review some of them. In a systemic review through the PubMed library, 57 FH-causing mutations were found in 17 countries from the MENA region. For example, the "Arabic allele" discovered in 2012 (*LDLR* c.1706-2A>T) is a single base-pair substitution mutation that leads to alternative splicing which deletes nucleotides. It was first discovered among Arab tribes. The only FH-related variants identified in the Bahraini, Algerian, and Saudi Arabian

populations were (*LDLR* c.1706-2A>T), (*LDLR* p.E408K), and (*LDLR* c.2439G>A). *LDLR* p.G343C and *LDLR* c.2446A>T (a nonsense mutation) were initially identified in the Tunisian population. Al-Waili et al. (2012) were the first to report an FH-related mutation in *PCSK9* in Oman's Arab population, namely *PCSK9* p.V474I. There were no ApoB mutations identified in the MENA area; the majority were in *LDLR*, with few changes discovered in *PCSK9* and *LDLRAP1*. There were no FH-related mutations reported from Kuwaiti, Qatari, Emirati, Jordanian, Libyan, Yemeni, and Egyptian populations (Baltimore et al., 2015).

The Lebanese allele (*LDLR* c.2043C>A) refers a mutant *LDLR* that is shorter than average. This failure to produce a complete protein is caused by a single nucleotide change which results in a premature stop codon at amino acid 660, that removes 180 residues from the mature protein, leaving only a ligand binding domain and a partial EGF Precursor Homology Domain. This mutation was initially identified in four unrelated patients, three Lebanese and one Syrian, thus named the Lebanese allele (Lehrman et al., 1987).

CHAPTER THREE
(MATERIALS AND METHODS)

3.1 Subjects

The research study received approval from the ethics committee of Al-Quds University. Al-Mizan Specialty Hospital permitted us to visit their cardiology clinic in July/2023. Patients at Al-Mizan Hospital's cardiology clinic diagnosed with premature coronary artery disease were invited to participate in our study. The inclusion criteria: (1) fasting plasma LDL-C level >190 mg/dL and triglycerides < 220 mg/dL; (2) physical findings of tendon xanthomata/ xanthelasma/corneal arcus or premature CAD or a first degree relative with hypercholesterolemia compatible with an autosomal dominant inheritance manner. The presence of other causes for hypercholesterolemia, such as thyroid, renal or hepatic disease, and familial combined hyperlipidemia were considered as exclusion criteria. Seven patients were found to meet our criteria and accepted to be enrolled in our study; four participants were members of the same family (family X), and the other three participants were members of another family (family E). Each patient was given a code (FHX01, FHE02, FHE03, FHX04, FHE01, FHE02, FHE03).

A consent form was given to each participant to clarify the study process and have their acceptance for giving blood samples and for publication of the results (see appendix). Each participant completed a questionnaire to collect family and medical history (see appendix).

3.2 Blood samples

Whole blood was drawn in an EDTA-anticoagulant tube for DNA extraction, and a single blood tube was taken from each participant to obtain a serum sample. The samples were processed on the same day. After centrifugation (2000× *g*, 10 min at room temperature), serum was used for Lipid profile tests and whole blood was stored at -20°C before DNA extraction.

3.3 Lipid profile measurements

The concentration of plasma total cholesterol, High Density Lipoprotein Cholesterol (HDL-C), and triglycerides were done in the Consulting Medical Laboratory, Nablus, Palestine. LDL-C levels were calculated using the Friedwald formula (LDL-C= total cholesterol - HDL-C – (triglycerides/5)).

3.4 Whole exome sequencing (WES), including CNVs and mitochondrial genome

The WES test was carried out in the Molecular and Genetics lab of Istishari Arab Hospital. The procedure entailed collecting a blood sample from the patient with the highest LDL-C level, followed by Whole Exome Sequencing (WES), which included an assessment of CNVs and mitochondrial genome. DNA was measured with Qubit v.3, and its quality was confirmed using gel electrophoresis.

The TruSeq Capture Exome Kit (Illumina) was utilized to prepare the library, which covered 45 Mb of exonic material. The probe set was created to enrich 214,405 exons. The data were then sequenced on NextSeq 500, and the reads were mapped to the reference human genome (h19) using a BWA aligner. Before calling variants using GATK (Genome Analysis Toolkit), the BAM-formatted mapped reads went through multiple preprocessing stages, including removing PCR duplicates, realigning around indels, and recalibrating base quality.

The ultimate list of variations was annotated using ANNOVAR and information from multiple databases, including minor allele frequency (MAF) data from PopFreqMax and variant effect predictors such as SIFT, PolyPhen-2, and REVEL.

To remove less trustworthy variations, which were with low coverage, synonymous alterations indicated to be benign by SIFT, PolyPhen-2, and REVEL, MAF > 1% on gnomAD, PopFreqMax, and our Palestinian in-house database were excluded.

3.5 Primer design

According to WES findings, Forward primer was ordered as published by Yang et al. 2007 (Yang et al., 2007) and Reverse primer was designed for the segregation analysis and Sanger sequencing of the LDLR gene exon 9 using Primer3 software. LDLR gene exon nine was amplified by using the primer:

Exon9F: GAGGCACTCTTGGTTCCATC; Exon9Rnew: GCTCACCTGCAGATCATTC; that amplifies 200 bp.

3.6 DNA extraction

Genomic DNA was extracted from EDTA whole blood with Genomic DNA Mini Kit (Blood/Cultured cell) following to the manufacturer's (Geneaid and Epicenter) directives. By using NANODROP spectrophotometer, DNA's purity and concentration were measured for all participants, and DNA purity was >1.7.

3.7 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) amplification was performed in accordance with the procedure, utilizing the designed primer and extracted DNA samples. PCR was performed in a 25-microliter reaction volume with the PCR-Biosystems Ready mix (2X PCRBIO HS Taq Mix Red, Jerusalem, Israel) and the BIO-RAD PCR-system T100 thermal cycler. Around 90 nanograms of eluted DNA from the blood sample were used in the PCR. The reaction conditions included a 5-minute pre-denaturation phase at 95°C, followed by 30 seconds of denaturation at 95°C, 30 seconds of annealing at 55°C, and 60 seconds of extension at 72°C, which was repeated 34 times. To serve as a PCR-negative control, a reaction buffer containing no human DNA was added with the samples.

3.8 Agarose gel electrophoresis

The amplicon for the characterized gene was electrophoresed on 2% agarose gels (SeaKem® LE agarose gel) at 100 V in 1x Tris-Acetate-EDTA buffer (TAE) with 8-microliter of ethidium bromide and visualized under UV light using a gel documentation system (the Bio-Imaging Systems MiniLumi transilluminator). To determine the molecular weight of the amplicon, a 50 bp molecular weight standard ladder (ThermoScientific GeneRuler) was employed as a reference for each gel.

3.9 Sanger sequencing

All PCR products visible by gel electrophoresis were sent for Sanger Sequencing at Hylabs in Jerusalem, Israel. The chromatograms were verified, and the sequences were assembled using BioEdit software.

3.10 Statistical analysis

Data was coded and entered to be analyzed using Statistical Package for Social Sciences (SPSS) version 23. Descriptive statistics were used to describe our sample's mean age, mean LDL-C level, skewness, percentages of gender, smoking status, statin use and angiography procedure. Crosstabulation and chi-square were used to obtain the relationship between our variants. We used the ANOVA method to test the significance of the relationship between LDL-C and sequence results.

CHAPTER FOUR
(RESULTS)

4.1 FH proband

We found that seven patients from the clinic we visited fulfilled our inclusion criteria. They were members of two families: four members of family X and three of family E. We selected our proband from family E depending on LDL-C since he has the highest LDL-C level.

4.1.1 Medical history

FHE01 is a 44-year-old male with a known case of premature CAD. In 2014, he was evaluated as a case of acute myocardial infarction (AMI) and referred for cardiac angiography and intervention. He underwent five cardiac angiographies with stent implantation in 3 coronaries. He underwent coronary artery bypass grafting surgery (CABG) for three vessels two years ago. On his first admission as a case of AMI, his lipid profile was abnormally high since his LDL-C level was 330 mg/dL and 232 mg/dL on his last admission despite being on high-intensity statin therapy. He has a strong family history of premature CAD among his father and all of his brothers. On physical examination, he lacked the signs of hypercholesterolemia, such as xanthomas and xanthelasmas. This patient fulfilled our inclusion criteria and was chosen as our study proband.

4.1.2 Whole exome sequence (WES) result

The WES report showed that our proband has a heterozygous, likely pathogenic, missense NM_000527.2: c.1210A>G p. (Thr404Ala) variant in exon 9 of the *LDLR gene*. The genetic diagnosis of heterozygous familial hypercholesterolemia had been made. No other cholesterol metabolism-responsible genes (*PCSK9 and ApoB*) are affected.

4.1.3 c.1210A>G p. (Thr404Ala) variant interpretation

We analyzed the exon9 sequencing file and confirmed the pathogenicity of this variant using the Franklin website (https://franklin.genoox.com/clinical-db/variant/snp/chr19-11223977-A-G?app=acmg-classification). This variant causes a single base pair substitution that alters the genetic code and produces a different amino acid in the sequence. This variant substitutes adenosine for guanine, substituting the amino acid threonine for alanine at position 404 in the YWTD-1 region in the EGF Precursor Homology Domain of LDLR protein.

4.2 PCR and Sanger sequencing for the seven found patients.

4.2.1 PCR and gel electrophoresis

After the WES result, we designed a primer and performed PCR for the other six patients who fulfilled our inclusion criteria and our proband as a positive control.

4.2.2 Sanger sequencing

The PCR products were sent for sanger sequencing. Members of family X were negative for c.1210A>G p. (Thr404Ala) variant. On the other hand, members of family E were positive for the variant which directed us towards continue cascade screening for this family. Table (5) shows the sequence results for both families. Figure (11) and figure (12) represents multiple sequence alignment for both families and chromatograms for 3 selected participants, respectively.

4.3.2 Sanger sequencing of PCR products

Family E members' PCR products were sent for Sanger sequencing. Results showed that the proband's father has the variant in homozygous genotype (G/G) and had transmitted it to all his sons. That is why we enrolled his brothers into the study, and two out of three also have the variant

in a homozygous genotype. The proband's nephews and nieces were enrolled on the study as their fathers were affected, and his cousins were enrolled since their fathers were also affected. Table (6) shows the genotyping of participating family E members.

Table 5: Sanger sequence results of exon9 of LDLR.

PCR code	Sequence result
FHX01	A/A*
FHX02	A/A
FHX03	A/A
FHX04	A/A
FHE01	A/G**
FHE02	A/G
FHE03	A/G

*A/A: Wildtype, **A/G: Heterozygous c.1210 A>G substitution.

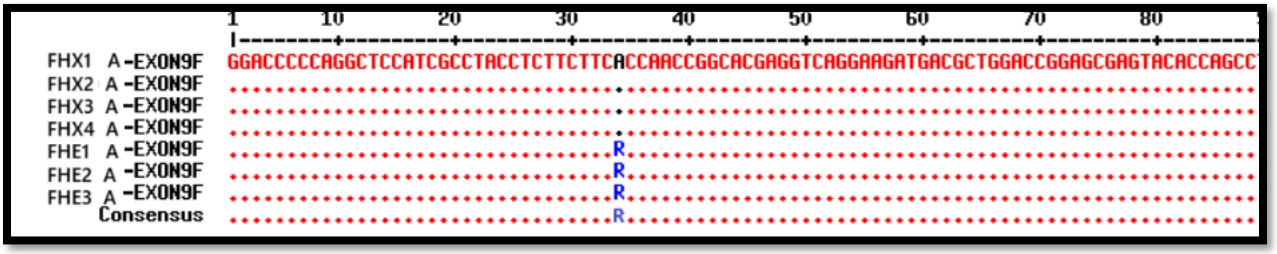
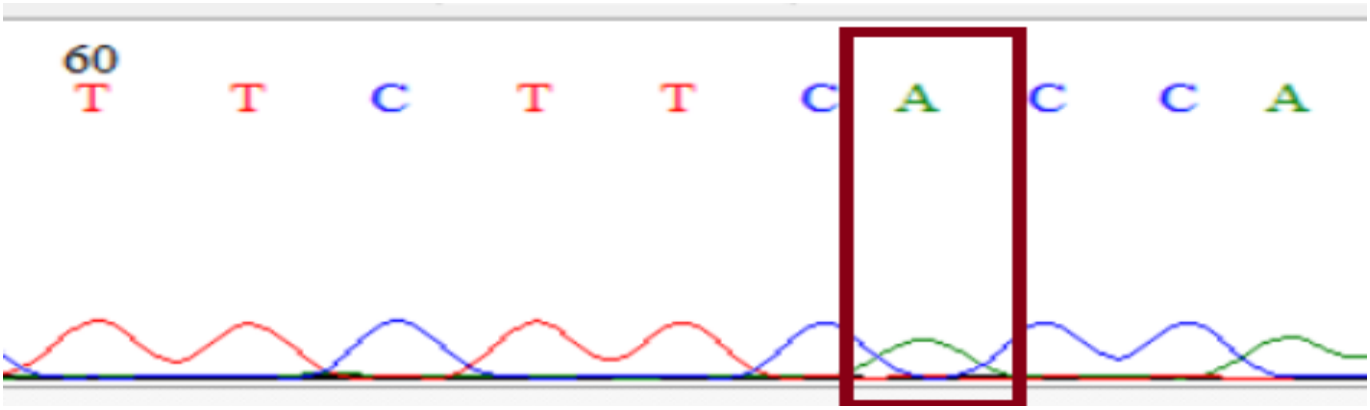
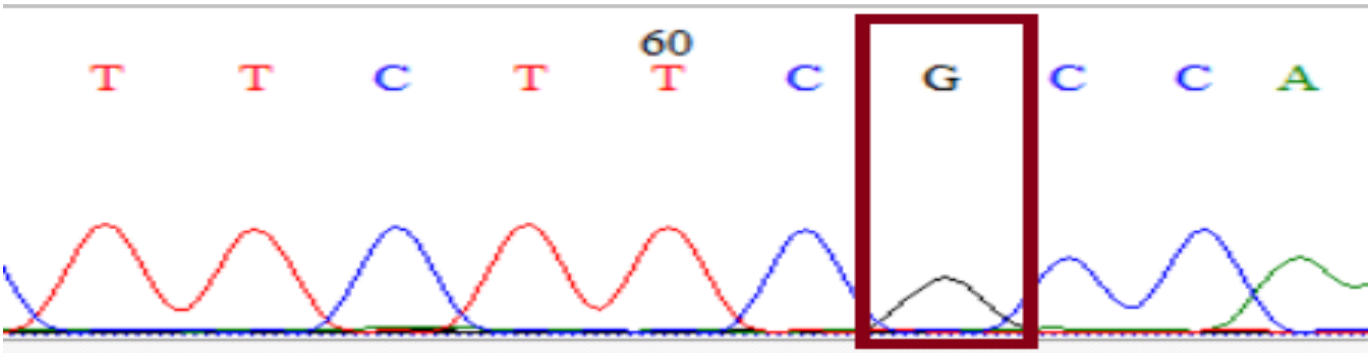


Figure (11): Multiple sequence alignment for family X and family E members shows that family X members are normal while family E members have heterozygous c.1210 A>G substitution. A: Wildtype, R: Heterozygous.

1-



2-



3-

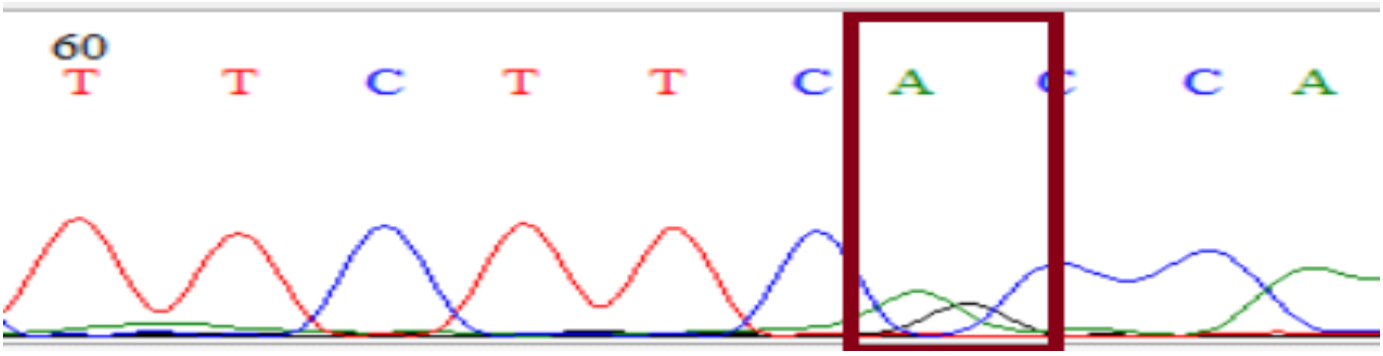


Figure (12): Representative chromatograms for 3 selected participants: 1- wildtype AA, 2-homozygous GG, 3-heterozygous AG.

Table 6: Sequence result for PCR products of family E members

PCR code	Sequence result	PCR code	Sequence result
FHE04	G/G***	**FHE22**	A/A
FHE05	A/A*	**FHE23**	A/A
FHE06	A/G**	**FHE24**	A/A
FHE07	A/G	**FHE25**	A/A
FHE08	A/G	**FHE26**	A/A
FHE09	A/G	**FHE27**	A/A
FHE10	A/G	**FHE28**	A/A
FHE11	A/G	**FHE29**	A/G
FHE12	A/A	**FHE30**	G/G
FHE13	A/A	**FHE31**	A/G
FHE14	A/G	**FHE32**	A/G
FHE15	G/G	**FHE33**	A/G
FHE16	G/G	**FHE34**	A/G
FHE17	G/G	**FHE35**	G/G
FHE18	G/G	**FHE36**	A/A
FHE19	G/G	**FHE37**	A/A
FHE20	A/A	**FHE38**	A/G
FHE21	A/A		

*A/A: Wildtype, **A/G: Heterozygous c.1210 A>G substitution, ***G/G: Homozygous c.1210 A>G substitution.

4.4 Family pedigree

After we had the sequence results for our sample, we could draw the family pedigree for FH in family E members who participated in our study. Figure (13) shows the pedigree. We also constructed a pedigree for family X as shown in figure (14).

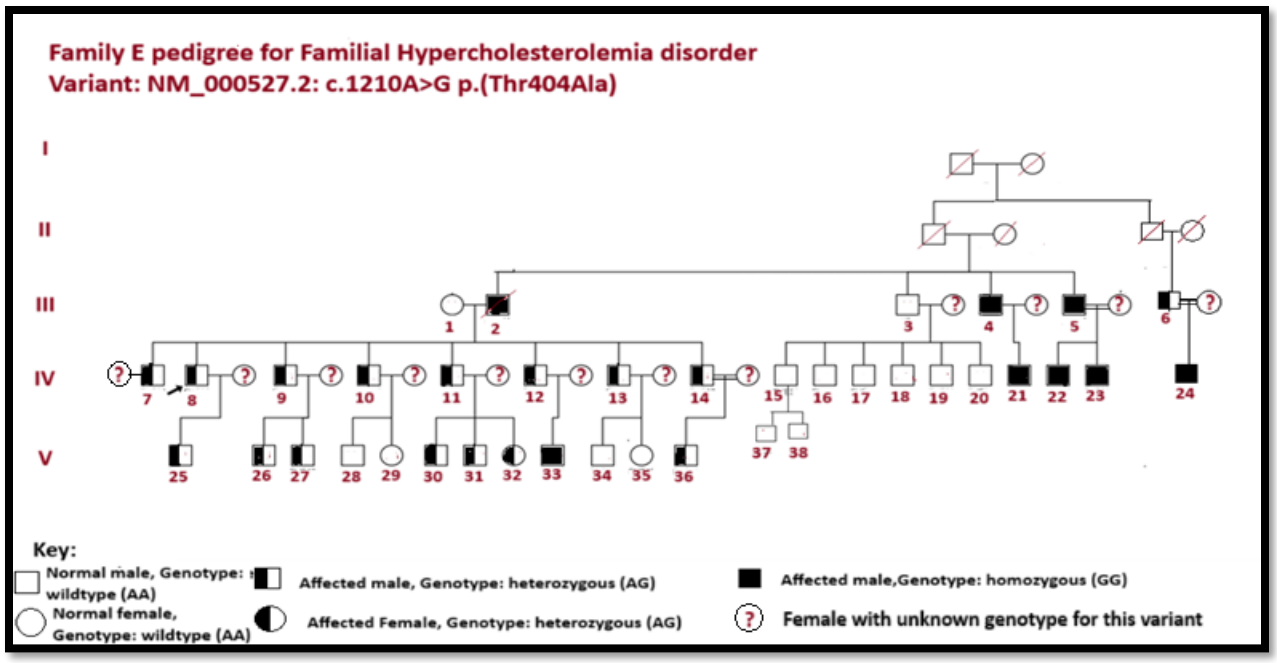


Figure (13): Family E pedigree for FH according to cascade screening.

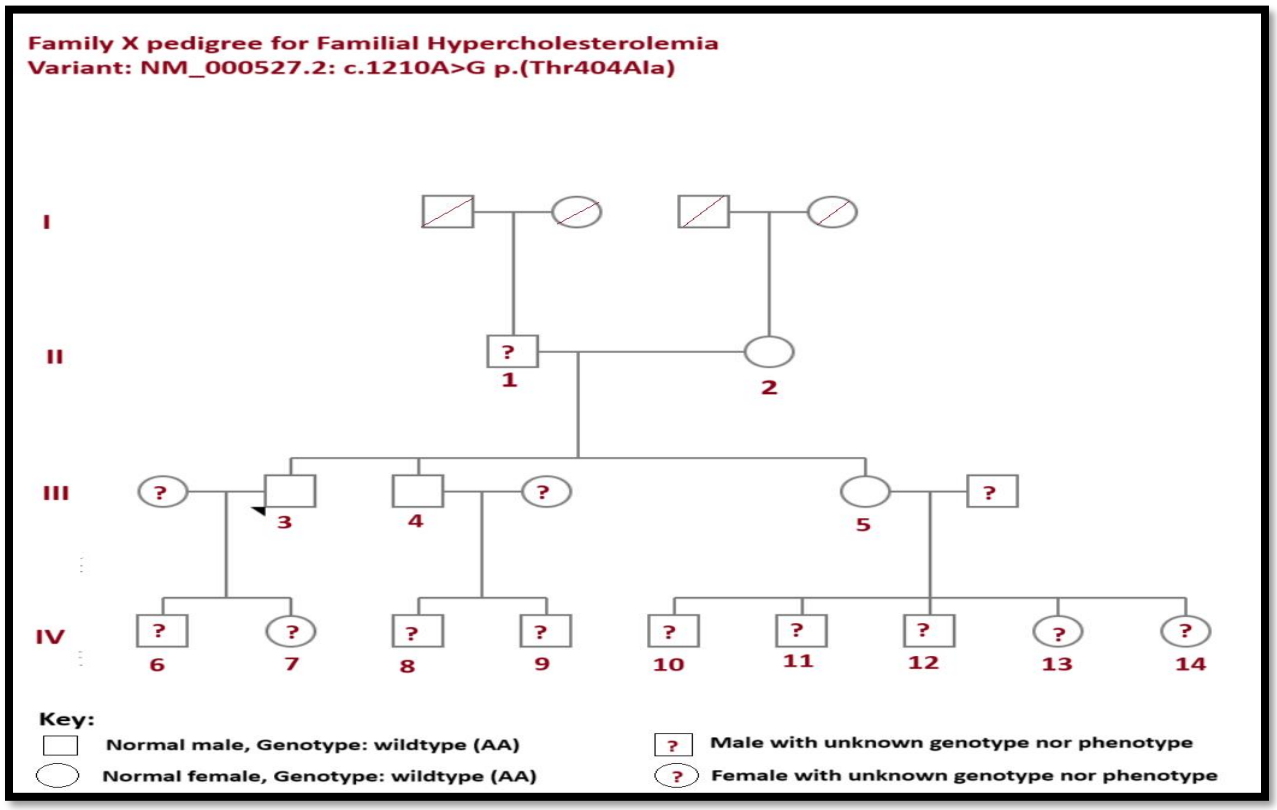


Figure (14): Family X pedigree for FH.

4.5 Statistical analysis

4.5.1 Study population

During October 2023, an extra 35 samples were collected from family E, making our sample size 38. Medical histories were obtained using questionnaires and medical records. 89.5% were males, and the mean age was 37. All study population has a family history of premature CAD, and 37.8% have a personal history of premature CAD. All the participants with a personal history of premature CAD had recurrent cardiac events. None of our participants had physical findings of hypercholesterolemia, such as xanthoma and xanthelasma. 51.4% were smokers. Only 35% of our participants take lipid-lowering agents like statin.

4.5.2 Lipid profile

2 EDTA blood tubes and one plain tube were collected from each participant. The plain tube was sent for lipid profile measurement. One participant was excluded from data analysis as his triglyceride level was >220 mg/dL, which gave a false LDL-C level. The mean LDL-C level was 129.5, the mean triglycerides level was 150 mg/dL, and the mean total cholesterol was 194, all of which reflect the high levels of lipid profile in this family. All variables were not distributed normally among the study population except for age, which reflects that there are abnormally high or low values. Table (7) represents descriptive statistics of the variables we used in statistical analysis.

The participants were subcategorised into five categories of LDL-C level: optimal (<100 mg/dL), near-optimal (100 to 129 mg/dL), borderline high (130 to 159 mg/dL), high (160 to 189 mg/dL) and very high (190 mg/dL and higher). Table (8) shows frequencies and percentages of each category. 43.2% of the participants fell in the abnormal high categories (borderline high, high and very high), and around one-third of them were in the very high category.

Despite around half of our sample falling in the abnormally high categories, only 35% use lipid-lowering agents, like statins. The mean LDL-C of those 35% who take statin was 153 mg/dL and 117 mg/dL among the remaining 65% who do not take statin. Using ANOVA, no significant relationship was found between statin use and LDL-C level.

Table 7: Descriptive statistics of study population

	N	Min. value	Max. value	Mean	St.deviation	Skewness
Age	38	10	76	37.08	17.872	0.447
LDL-C	37	44.2	293.2	129.589	64.6	0.692
HDL-C	37	19	65	34.49	10.514	1.315
Total cholesterol	37	100	350	194	63.141	0.661
Triglycerides	37	35	*341*	150.57	63.369	1.190

Table 8: LDL-C categories

LDL-C category	Number of participants	Percentage
Optimal	14	37.8%
Near-optimal	7	18.9%
Borderline high	3	8.2%
High	7	18.9%
Very high	6	16.2%
Total	37	100%

4.5.3 LDLR sequencing

Sanger sequencing of exon 9 in the LDLR gene revealed that 37.8% of our sample were genotyped A/A, 40.5% were genotyped A/G, and 21.6% were genotyped G/G. Crosstabulation and chi-square were used to test the relation between *LDLR* exon9 sequencing and LDL-C category, history of angiography, intervention after angiography, and family and personal history of premature CAD. There was a significant relationship between the LDLR sequence result, personal history of premature CAD, and LDL-C category, but not family history of premature CAD. This test found that sequence result affected the presence of a personal history of premature CAD and is a causative variant of elevated participants' LDL-C plasma levels. Table (9) clarifies the significance of these relationships.

By using compare means, LDL-C level and *LDLR* sequence result showed a significant relationship (P= 0.000); individuals who genotyped G/G had a mean LDL-C of 203 mg/dL, A/G had a mean of 138 mg/dL, and A/A had a mean of 79 mg/dL. This result further implies that the LDLR sequence, which affects the level of plasma LDL-C, is pathogenic and causes hypercholesterolemia.

4.5.4 DLCN criteria and LDLR sequence

DLCN score was calculated for each participant who does not take a statin (twenty-four participants) and was subcategorized into unlikely if DLCN score<3, possible (3-5), probable (6-7) and definite if DLCN score ≥8. Then, its relationship to the LDLR sequence was tested using ANOVA and chi-square; a significant relationship was found. Table (10) summarizes frequencies of DLCN score category within *LDLR* sequence groups. Despite the significant relationship between the DLCN score category and *LDLR* sequence, eight out of twenty-one participants with a DLCN score < 3 (unlikely to have FH) were genetically positive for FH.

Table 9: *LDLR* exon 9 sequence result and its relation to multiple variables.

		LDLR exon 9 sequence result						Total	P value <0.05
		A/A	%	A/G	%	G/G	%		
Family Hx of P.CAD	Yes	14	37.8%	15	40.5%	8	21.7%	37	0.430
	No	0	0%	0	0%	0	0%	0	
Personal Hx of P.CAD	Yes	1	7%	8	57%	5	36%	14	0.010
	No	13	56.5%	7	30.4%	3	13.1%	23	
Hx of angiography	Yes	3	20%	8	53.3%	4	26.7%	15	0.179
	No	11	50%	7	31.8%	4	18.2%	22	
Intervention after angiography	No angiography	11	50%	7	31.8%	4	18.2%	22	0.365
	No intervention	2	50%	1	25%	1	25%	4	
	Stents	0	0%	5	71.4%	2	28.6%	7	
	CABG	1	25%	2	50%	1	25%	4	
LDL-C categories	Optimal	11	78.5%	2	14.3%	1	7.2%	14	0.000
	Near optimal	2	28.6%	5	71.4%	0	0%	7	
	Borderline high	0	0%	3	100%	0	0%	3	
	High	1	14.3%	4	57.2%	2	28.5%	7	
	Very high	0	0%	1	16.7%	5	83.3%	6	
LDL-C mean level		79 mg/dL		138 mg/dL		203 mg/dL			0.000

Hx: History, P.CAD: Premature coronary artery disease.

Table 10: Frequencies of DLCN score category within *LDLR* sequence groups.

DLCN score category						Total
		Unlikely	Possible	Probable	Definite	
LDLR sequence	A/A	13	0	0	0	13
	A/G	7	0	0	0	7
	G/G	1	1	2	0	4
Total		21	1	2	0	24

CHAPTER FIVE
(DISCUSSION)

FH runs through families in an autosomal dominant manner. That means one affect parent can transmit the disease to his/her siblings and chance of passing it to siblings greatly increases if both parents are affected with FH-causing variant/s. Also, it is an atherogenic disease which dramatically increases the risk of premature ASCVD (Schmidt et al., 2020). So, it is imperative to examine any patient with elevated LDL-C and CAD closely and to screen his family for hypercholesterolemia in order to make an early diagnosis and offer early management. This approach could serve one of the significant health aims of decreasing the CVD burden.

The variant c.1210A>G p. (Thr404Ala) was confirmed in our proband. It substitutes the amino acid threonine for alanine at position 404 in LDLR protein. Three different variants were reported and submitted to the ClinVar database at this position: c.1210A>T p. (Thr404Ser), c.1210A>C p. (Thr404Pro) and c.1211C>T p. (Thr404Ile) but our proband's variant was not reported nor submitted to ClinVar database (Galicia-Garcia et al., 2020). So, this variant's clinical interpretation and functional characterization have not been described before.

Recently, in 2023, the NM_000527.5(*LDLR*): c.1210A>C (p. Thr404Pro) variant was classified as Uncertain significance - insufficient evidence for Familial Hypercholesterolemia according to the ClinGen Familial Hypercholesterolemia Expert Panel LDLR-specific variant curation guidelines (https://erepo.clinicalgenome.org/evrepo/ui/interpretation/893fa193-f63f-4fcd-b2be-1768f75bd99b). Also, the variant NM_000527.5(*LDLR*): c.1210A>T (p. Thr404Ser) was classified as Uncertain significance in April 2023 according to ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/431523/). As for the variant NM_000527.5(LDLR): c.1211C>T (p. Thr404Ile) was last evaluated in 2021 and is still classified in ClinVar database as Pathogenic/likely pathogenic variant (https://www.ncbi.nlm.nih.gov/clinvar/variation/251736/?oq=c.1211C%3ET[varname]+p.+(Thr404Ile)&m=NM_000527.5(LDLR):c.1211C%3ET%20(p.Thr404Ile)).

In our proband's variant, substituting threonine for alanine may alter the structure and function of the YWTD-1 repeat of the EGF Precursor Homology Domain. Threonine has a hydroxyl functional group, making it a polar uncharged amino acid. This amino acid can be phosphorylated by

threonine kinase. While alanine is a nonpolar uncharged amino acid that does not undergo phosphorylation. The different chemical properties of those two amino acids can affect the final structure and function of the protein.

The EGF Precursor Homology Domain is a main player in the release of lipoprotein and recycling of LDLR. The YWTD repeats; that form six-bladed beta propeller, are responsible for the release of lipoprotein as they displace it thereby acting like a substitutional substrate for the binding domain. Also, they act like sensors of pH which is the main factor of lipoprotein release. Another important function of YWTD repeats is to fold the LDLR correctly at cell surface. Most of the mutations occur here at this location are described as class 2 (partial or complete retention in ER) or class 5 (diminished receptor recycling capacity) (Galicia-Garcia et al., 2020). We conclude that this substitution can either destabilize the highly conserved structure of the YWTD repeats or interrupt the recycling process maintained by these repeats. In order to accurately predict the malfunction caused by this variant; a structural biologist should be consulted to build up the final structure of LDLR after replacing threonine by alanine and assume if this replacement affects the stability and function of the protein or not. Also, LDLR stability, LDLR expression and LDLR functional capacity which is represented as binding and uptake activities should be done. A significant relationship between LDLR exone9 genotype and LDL-C mean level, individuals who genotyped G/G had a mean LDL-C of 203 mg/dL, A/G had a mean of 138 mg/dL, and A/A had a mean of 79 mg/dL. This result further implies that the LDLR exon9 sequence result; which affects the level of plasma LDL-C, has a high suspicion of pathogenicity and can cause hypercholesterolemia.

Despite the mean of LDL-C level for family X was >190 mg/dL, their sequence results were negative for this variant. This can be attributed to another FH-causing variant running among this family or polygenic causes of hypercholesterolemia. 30% of patients with clinical FH may have a polygenic cause of hypercholesterolemia (Trinder et al., 2019). Polygenic hypercholesterolemia may present in a severe form depending on number of variants involved; the more SNPs involved, the more severe phenotype (Tandirerung, 2022). Among family X, phenotype was not in that severity and this may be attributed to the co-presence of LDL-C lowering variants. Polygenic risk score is a useful tool to determine the likelihood of having polygenic hypercholesterolemia but it is not applied in our health or research centers. WES can be done for one member of this family

to detect all variants that may be LDLR-raising or lowering, and if any variant is found, cascade screening should be continued to the whole family.

WES is a very precise process based on next-generation sequencing that has gained popularity in medical genetics. Most of the current mutation screening methods in FH field depend on focusing on the most important regions of known FH-related genes while Whole exome sequencing allowed us to analyze the full coding sequence of the gene (Futema et al., 2014). WES is standard to look for polygenic hypercholesterolemia as it gives a wider read not restricted to multiple FH-causing genes only. Also, another advantage of WES over targeted genes sequencing is that WES can identify variants in uncommon FH-causing genes and this decreases the false-negative results by using targeted genes sequencing. In the same way, the LDL-C lowering variants can be identified by WES which may give an explanation for less severe phenotypes of polygenic hypercholesterolemia (Athar et al., 2019).

Around 62% of participants form family E were positive for this variant, but only half of them have LDL-C level above 129 mg/dL (in the high LDL-C subcategories). The other half of the affected participants have normal and near normal levels of LDL-C. On one hand, this makes us think twice about the pathogenicity of the variant we found. On the other hand, our finding can be explained by their young age, reduced penetrance or expression of the variant, or simply by taking high-intensity statin regularly (Orringer & Grant, 2020). Also, lifestyle modification such as diet concerns and increase physical activity can give additional reduction in LDL-C level. This finding gives a clue that normal LDL-C level does not exclude the diagnosis of FH in a family with a known FH-causing running variant across generations and adds a weakness point to the clinical criteria of FH diagnosis. Thus, when an FH-causing variant is suspected among certain family, genetic testing is the gold standard diagnostic tool.

A significant relationship was found between LDLR exon9 sequence result and personal history of premature CAD and LDL-C which further implies the role of LDL-C in the development of ASCVD. On the other hand, no significant relationship was found between genotype and family history of premature CAD, history of angiography nor interventions after angiography. The lack of significance in the relation between sequence result and history of angiography and intervention after angiography can be referred to that not all participants underwent angiography as a diagnostic tool for CAD. Also, not all angiographies should be ended with intervention as intervention is

made only for significant coronary artery lesions that cause more than 50% stenosis in left main artery and more than 70% in other coronaries in most cases (https://radiopaedia.org/articles/coronary-artery-disease). Thereby, no intervention does not mean no coronary artery disease is present but means not significant lesions may be found. To test this relationship correctly, the exact angiographic findings should be used.

Speaking of coronary artery lesions, new methods have been implicated in assessing the significance of coronary artery lesions as add on for the angiographic findings such like fractional flow reserve (FFR). FFR is a reliable strategy to assess the hemodynamic significance of coronary artery atherosclerotic plaques and also gives a prognostic information. It measures the ratio of pressure distal to the stenosis in the coronary artery to the pressure of the aorta. Normal value is 0.94-1. Values below 0.75 should have certain intervention either by medications or revascularization depending on how low the FFR is (Clarke, Duarte Lau, & Zarich, 2020). Sanz Sa´nchez et al, found in 2023 that Patients with CAD who had FFR-guided percutaneous intervention (PCI) had a reduced risk of all-cause mortality and MI compared to those who received non-physiology-guided revascularization (Sanz Sánchez et al., 2023). The importance of this tool among FH population is translated into life-saving as defining the physiologically and hemodynamically significant lesions is critical for those patients. The coronary atherosclerotic plaques in FH patients have high risk of rupture characteristics. One of them is the high lipid necrotic core as FH patients have extremely high LDL-C levels and this enriches the plaque core with lipids which make it vulnerable for rupture, causing myocardial infarction and increasing morbidity and mortality (Clarke et al., 2020). So, depending on angiographic findings alone could miss some physiologically significant lesions and leaving them without revascularization may expose them for rupture and causing complications. Cardiac centers should be aware to these facts.

Talking about statin use, no significant relationship was found between statin use and LDL-C level and category. Many factors can interplay; only 35% of our participants take statin as a lipid lowering agent. Even those who take statin could be not adherent to medication or having low or moderate- intensity statin instead of high-intensity statin regimen which is recommended for FH and patients with history of premature CAD. Also, polygenic causes of hypercholesterolemia and compound mutation causing FH may result in incompetence of statin in lowering LDL-C. another reason for incompetence of statin is hyper Lp(a). In individuals with hyperLp(a), insufficient

decrease in LDL-C concentration might be attributed to the presence of Lp(a)-cholesterol in LDL-C. In general, statins do not appreciably change Lp(a) readings (Santos, 2023).

International guidelines share the strategy of aggressive treatment to reduce ASCVD risk. They all agreed to treat FH using diet and pharmacotherapy to achieve ≥50% LDL-C reduction from baseline, LDL-C <100 mg/dL or <70 mg/dL in diabetics for primary prevention and LDL-C 70 mg/dL or <55 mg/dL in diabetics for secondary prevention. Children with FH should be counselled for management starting from the age of 8 years and LDL-C goal is <135 mg/dL at age >10 years. High-intensity statin regimens are the first line of FH management. If treatment goal was not achieved or high-intensity statin was not tolerated by the patient, physician can add ezetimibe. Again, if treatment goal was not achieved, bile acid sequestrants or PCSK9 inhibitors can be added. Lomitapide is an inhibitor of the microsomal triglyceride transport protein that can be used in the treatment of FH. The microsomal triglyceride transport protein plays a role in VLDL-C assembly and secretion of apoB-containing VLDL-C. Inhibition of this protein disturbs the VLDL-C formation which is modified to LDL-C by LPL and HL and this decreases LDL-C levels (Rosenson, 2021). Unfortunately, PCSK9 is extremely expensive and not routinely available in our country and same for limitapide. Pharmaceutical companies gave promises to provide and certify PCSK9 inhibitors soon for the Palestinian patients.

For participants who do not take statin (65% of the participants), DLCN score was calculated based on DLCN clinical criteria for diagnosing FH. We found that 38% of the 'unlikely to have FH group' was diagnosed genetically with FH. This finding further implies that clinical criteria have a large false-negative window and physicians should not rely on them completely which returns us to the important role of genetic testing in FH diagnosis. We calculated DLCN score only for participants who do not take statin as statin use lowers LDL-C which in turn lowers DLCN score and increases the false-negative possibilities. If we decided to include participants who take statin, we should use their first LDL-C level result before having statin, or correct LDL-C for statin and this needs more information about dosage and adherence to medication; which both were not possible to have.

All the participants with personal history of premature CAD had recurrent attacks of cardiac events despite aspirin and statin therapy. Non-adherence to treatment and other lipid metabolism disorder like elevated Lp(a) could be the contributing factors of cardiac events recurrence despite treatment.

Some studies revealed that elevated Lp(a) level above 40 mg/dL is associated with increased risk of ASCVD irrespective of LDL-C level, but others showed that this association is present only when LDL-C is high and vanished when LDL-C is low. In a recent investigation of CAD patients with LDL-C < 35 mg/dL, Lp(a) levels were found to be independently linked with major adverse cardiac events (MACE) and recurrent MI in patients with well-controlled LDL-C. Additionally, elevated Lp(a) in the presence of high LDL-C increases the risk of recurrent cardiac events and this is thought to be attributed to defect in Lp(a) catabolism. It is thought that part of Lp(a) catabolism is mediated through LDLR, since high LDL-C levels occupy the receptors, Lp(a) level is increased and its biological effect is enhanced. An interesting fact about Lp(a) is that its level is tend to be constant through life, but a meta-analysis showed that Lp(a) level increased after statin therapy. Thereby, monitoring Lp(a) level after statin therapy is more beneficial than baseline Lp(a) in predicting additional cardiovascular risk in FH patients (Zhu et al., 2022).

As mentioned before, FH is an autosomal dominant highly-atherogenic disorder, could. Marriage of both HeFH patients gives a chance for transmitting a homozygous mutation to one or more of their siblings which may have an extremely severe FH phenotype and very premature CAD (during the second decade of life). This emerges the importance of genetic counselling in the limitation of inherited diseases transmission among generations. This aim could be achieved by a wide campaign of CAD and FH awareness targeting all society strata; healthcare workers, teachers, engineers, students, etc, and most importantly, the patients who are having these two major health problems. Physicians should be alert to refer any patient with hypercholesterolemia and premature CAD for genetic counselling. These simple steps could make a big difference in decreasing the burden of cardiovascular disease in our population.

CHAPTER SIX

(CONCLUSION, RECOMMENDATIONS, LIMITATIONS AND FUTURE PERSPECTIVES)

6.1 Conclusion and recommendations

Putting all together, FH is underdiagnosed and undertreated in our population and this increased the number of premature ASCVD cases. Cascade screening is a beneficial and cost-effective process for diagnosis and treatment of FH early in life using lipid lowering agents in order to decrease the burden of ASCVD and prevent premature cardiovascular death among our population. The variant c.1210A>G p. (Thr404Ala) was not reported before and has a high suspicion of pathogenicity as its presence correlated significantly with LDL-C mean level in our sample.

In order to increase the rate of FH diagnosis, we recommend lipid profile screening for any individual with a family history of premature CAD and/or hypercholesterolemia. Also, we recommend genetic counselling for any patient with premature CAD with hypercholesterolemia that is running in families.

Genetic diagnosis by WES proved it is the gold standard investigation to diagnose FH as we proved that clinical criteria would underestimate cases number by ignoring individuals with normal LDL-C despite they would be genetically diagnosed with FH.

6.2 Limitations

The main limitations of this study are represented in time, fund and the stigma of inherited disease in our society. If more time was available, more participants would be asked to join the study in order to be able to generalize the study among the Palestinian population. Regular fund would give us more space to investigate the clinical significance of the variant we confirmed by measuring LDLR expression and function in our sample. Also, if more fund was available, we would send

another sample that we doubt it has another variant for WES. Another important point, families in our society still think of the inherited diseases stigma and this decreased the size of the sample especially females.

6.3 Future perspectives

We are planning to consult a structural biologist in order to confirm the effect of the variant we found on the final structure and function of LDLR. Then, after confirming the pathogenicity of this variant, we will continue the cascade screening for family E in order to diagnose as FH cases as we can. We also intend to investigate more families from different regions in Palestine for this variant and if possible, for other universally confirmed variants. Our team is preparing a community-based lectures to light the importance of genetic diseases screening and change the general outlook of genetic diseases as a stigma that should not be discovered.

CHAPTER SEVEN

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APPENDIX

Appendix (1): Consent form.

نموذج موافقة

جامعة القدس
Al-Quds University

أنا الموقع أدناه (الاسم) ---------- وحامل رقم هوية ----------
أقر بأنه تم إيضاح الدراسة الجينية في مرض ارتفاع الكوليسترول الوراثي وبأني أوافق على المشاركة طوعا في هذه الدراسة, وأقر بأني أتفهم احتمالية إصابتي أو حملي لصفة هذا المرض الجيني الذي يتم تشخيصه عن طريق إجراء فحص جيني للماده الوراثية المستخرجة من عينة دم, وبناء على ذلك أمنح موافقتي لاستخدام معلوماتي الشخصية والطبية وعينة من دمي لإرسالها إلى المختبر الجيني في المستشفى الاستشاري لعمل الفحوصات الجينية اللازمة ولا مانع لدي من نشر هذه النتائج لأغراض البحث العلمي.

وأقر بأنه قد تم إيضاح ما يلي من قبل القائمين على هذه الدراسة:

- الأمراض الجينية قد يتم توارثها عبر أفراد العائلة وبأن نتيجة فحوصاتي الجينية قد تؤثر على أفراد عائلتي.
- في حالة البحث عن طفره جينية, تحديدها بشخص المرض وعدم تحديدها لا ينفي احتمالية الإصابة بهذا المرض الجيني.
- أستطيع أنا المشارك في الدراسة التراجع عن قراري في المشاركة بأي وقت دون وجود عواقب ودون إعطاء الأسباب.
- سيتم ترميز عيناتي البيولوجية بحيث يكون الشخص الوحيد القادر على الاطلاع على نتائج فحوصاتي هو القائم على الدراسة ومشرفه المباشر.
- من الممكن إيجاد نتائج غير متوقعة لفحوصاتي, وعليه أود معرفتها: ☐ نعم ☐ لا

وعلى ذلك أوقع

توقيع المشارك في الدراسة:---------- التاريخ:----------

أنا ايناس سراحنه, طالبة الماجستير القائمة على دراسة:

"Detection of familial hypercholesterolemia variants in selected patients with premature coronary artery disease from Hebron region"

أقر بأن المشارك في الدراسة الموقع أعلاه على دراية كاملة بتفاصيل هذه الدراسة وقد منحنا الإذن باستخدام معلوماته الشخصية والطبية وعينة من دمه في هذه الدراسة. وعلى ذلك أوقع.

توقيع الطالب:---------- التاريخ:----------

Appendix (2): Questionnaire.

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استبيان لدراسة:

الكشف عن المتغيرات الجينية المسببة لمرض ارتفاع كولستيرول الدم الوراثي لدى المرضى المصابين بمرض تصلب الشرايين التاجية المبكر في منطقة الخليل

نحن مجموعة باحثين ضمن برنامج الماجستير في الأحياء الجزيئية والكيمياء الحيوية في جامعة القدس, نقوم بعمل هذه الدراسة التي سيتم نشرها على شكل رسالة ماجستير كمتطلب للحصول على هذه الدرجة العلمية.

تهدف هذه الدراسة إلى تحديد التغيرات الجينية لدى المرضى المصابين بارتفاع الكولستيرول في الدم وإثبات إن كان هذا الارتفاع هو السبب المؤدي الى الإصابة بمرض الشريان التاجي المبكر.

من خلال الإجابة على هذا الإستبيان، فإنك توافق على المشاركة في هذه الدراسة وتمنحنا الإذن بالرجوع إلى سجلاتك الطبية إن توفرت, وتوافق على نشر المعلومات المستخلصة من الفحوصات التي ستقوم بها. ونتعهد نحن القائمون على الدراسة بالتعامل مع معلوماتك بسرية تامة واستخدامها لأغراض البحث العلمي فقط بحيث لن يتم نشر او إيضاح أي معلومة قد تتسبب في معرفتك أو معرفة أي من معلوماتك الشخصية.

الرجاء الإجابة على الأقسام الثلاثة الأولى من هذا الاستبيان وترك القسم الرابع حتى تقوم بتعبئته بعد الرجوع إلى سجلاتك الطبية.

القسم الأول: المعلومات الشخصية

الرجاء إدخال المعلومات الآتية.

1.1 الرجاء تزويدنا بما يلي:

رقم الهوية: _____

رقم الهاتف: _____

البريد الإلكتروني: _____

2.1 الجنس:

☐ ذكر ☐ أنثى

3.1 تاريخ الميلاد: _______________ العمر: _____

4.1 مكان السكن: _____

5.1 الطول (سم): _____

6.1 الوزن (كغم): _____

7.1 هل أنت مدخن/ة؟
☐ نعم ☐ لا

8.1 هل تمارس/ين أي أنواع من الرياضة أو أي نشاط جسدي؟ إذا أجبت بنعم الرجاء تحديد المده الزمنية للنشاط .
☐ نعم ☐ لا

_____

****

القسم الثاني: التاريخ الطبي

1.2 هل تعرضت لجلطات قلبية سابقا؟
☐ نعم ☐ لا
-إذا كانت الإجابة نعم, كم كان عمرك؟
الرجاء ذكر التدخلات العلاجية التي تم اتباعها لعلاجك أن وجدت: (قسطره قلبية مع زرع دعامات شريانية/عملية قلب مفتوح مع زرع وصلات شريانية). _____

2.2 هل تعرضت لجلطات دماغية سابقا؟
☐ نعم ☐ لا
-إذا كانت الإجابة نعم, كم كان عمرك؟
الرجاء ذكر التدخلات العلاجية التي تم اتباعها لعلاجك إن وجدت. _____

3.2 هل سبق أن حدث امساد أو تضيق في الشرايين الطرفية (شرايين الساق أو الذراع أو البطن)؟
☐ نعم ☐ لا
-إذا كانت الإجابة نعم, كم كان عمرك؟
الرجاء ذكر التدخلات العلاجية التي تم اتباعها لعلاجك إن وجدت. _____

4.2 هل تعاني من تشوهات خلقية في القلب أو الشرايين القلبية (التاجية)؟
☐ نعم ☐ لا
-إذا كانت الإجابة نعم, الرجاء ذكر نوع التشوه الخلقي. _____
5.2 هل لديك أي من الأمراض الآتية ؟

☐ الضغط ☐ الأمراض الكلوية

☐ أمراض القلب والأوعية الدموية ☐ ارتفاع الكولسترول في الدم

☐ سكري ☐ الأمراض الخبيثة. ما نوع المرض الخبيث إن وجد؟ _____

☐ فرط نشاط أو كسل في الغده الدرقية ☐ مشاكل في العينين والنظر

☐ تشنجات أو نوبات صرع ☐ تأخر في النمو

☐ غير ذلك. _____

6.2 الرجاء ذكر الأدوية التي تأخذها لأي حالة صحية تعاني منها.

_____

_____

7.2 هل لديك أي زوائد دهنية تحت الجلد في الذراعين أو حول العينين؟
☐ نعم ☐ لا

****

القسم الثالث: التاريخ المرضي العائلي

1.3هل هناك أفراد في عائلتك مصابين بمرض ارتفاع الكولسترول في الدم؟

☐ نعم ☐ لا

2.3هل هناك أفراد في عائلتك تعرضوا لجلطات قلبية قبل سن 45 عام للرجال و 55 عام للنساء؟

☐ نعم ☐ لا

-إن كانت الإجابة نعم, الرجاء تحديد صلة القرابة مع المريض _____
و الإجراء التداخلي الذي تطلبته حالة المريض (قسطره قلبية مع زرع شبكيات/عملية قلب مفتوح مع زرع وصلات للشرايين القلبية (التاجية)) _____

3.3 هل هناك أفراد في عائلتك تعرضوا لجلطات دماغية أو طرفية قبل سن 45 عام للرجال و55 عام للنساء؟

☐ نعم ☐ لا

-إن كانت الإجابة نعم, الرجاء تحديد صلة القرابة مع المريض _____
و الإجراء التداخلي الذي تطلبته حالة المريض _____

4.3 هل هناك أفراد في عائلتك لديهم زوائد دهنية تحت الجلد أو حول العينين؟

☐ نعم ☐ لا

****

القسم الرابع: الفحوصات الطبية

سيتم ملئ هذا الجزء من الاستبيان من قبلنا بعد الرجوع إلى سجلاتك الطبية .

1.4مستوى الكولسترول: _____

2.4 مستوى الدهون الثلاثية: _____

3.4 مستوى LDL: _____

4.4 مستوى HDL: _____

5.4 مستوى السكر التراكمي HbA1C : _____

توقيع الباحث: _____ التاريخ: _____

شكرا جزيلا

Appendix (3): Ethical approval.

Al-Quds University
Faculty of Medicine
Abu-Dies, Jerusalem

جامعة القدس
كلية الطب
أبوديس – القدس

Research Ethics subcommittee of Faculty of medicine

Letter of Ethical approval

Date:7/6/2023

Ref#: R2-7-23

Dear Applicants: Dr. Kifaya Suleiman and Ms. Enas Sarahna

Biochemistry and Molecular Biology master program

The Research Ethics subcommittee of faculty of medicine has recently reviewed your proposal entitled "Detection of familial hypercholesterolemia genetic variants in selected patients with premature coronary artery disease from Hebron region". Your proposal is deemed to meet the requirements of research ethics subcommittee at Al-Quds University.

Note: This letter can be used to apply for the central Al-Quds University research ethics committee if needed

Best of luck,

Dr. Suheir Ereqat
Head of research ethics subcommittee
Biochemistry and Molecular Biology master program
Faculty of Medicine-Al-Quds University

P.O Box 20002
Tel 02-2799203, Fax 02-2796110

ص. ب 20002
هاتف 022799203 فاكس 022796110

الكشف عن المتغيرات الجينية المسببة لمرض ارتفاع كولسترول الدم الوراثي لدى المرضى المصابين بمرض تصلب الشرايين التاجية المبكرفي منطقة الخليل

إعداد: ايناس يوسف مصطفى سراحنه

إشراف: د.كفاية عزمي

الملخص بالعربية

ارتفاع كوليسترول الدم الوراثي هو مرض يتم توارثه بالطريقة السائدة لنقل الأمراض الجينية, وهو ناتج عن طفرات في الجينات المسؤولة عن أيض الكوليسترول (البروتين الدهني قليل الكثافة او الكولسترول السيء LDL-C) وهم: LDLR, ApoB و PCSK9 في أغلب الحالات المرضية, مما ينتج خلل في التخلص من هذا النوع من الكولسترول فيزداد مستواه في الدم. 85-90% من هذا المرض يكون نتيجة لطفرة جينية في الجين المسؤول عن انتاج مستقبل هذا النوع من الكولسترول (LDLR). قد يتم توارث هذا المرض بطريقة متجانسة; أي أن كلا الأليلين يحمل الطفرة, أو غير متجانسة; أي أن أليلاً واحدً يحمل الطفرة. يترواح معدل انتشار هذا المرض ما بين 1:400 – 1:192 حسب العرق. الخطر الكامن وراء الإصابة بمرض ارتفاع كولسترول الدم الوراثي هو ان هذا النوع من الكولسترول مسبب أساسي لمرض تصلب الشرايين.

الهدف من هذه الدراسة هو تحديد الطفرات المسببة لهذا المرض وتفعيل مبدأ الفحص المتتابع للحالات لدى العائلات التي يوجد بها مصابين بمرض تصلب الشرايين المبكر ويعانون من ارتفاع LDL-C في الدم.

لقد تم اختيار مريض تنطبق عليه معايير الاشتمال والتي هي: كولسترول الدم السيئ (LDL-C) >190 ملغم/ديسيلتر, يوجد لديه في الفحص السريري زوائد دهنية حول عينيه و قرب المفاصل أو لديه تاريخ عائلي أو شخصي بمرض تصلب شرايين القلب المبكر. تم إرسال عينة دم للمريض لإجراء فحص تسلسل الإكسوم الكاملwhole exome sequencing) , ثم تم سحب عينات دم من أقربائه بالدرجة الأولى لإجراء فحص PCR و sanger sequencing.

لقد تم إيجاد طفرة في الاكسون التاسع للجين المسؤول عن انتاج مستقبل LDL-C (LDLR) وهي NM_000527.2: c.1210A>G p. (Thr404Ala), لم يتم الإبلاغ عن هذه الطفرة من قبل. ثم تم تطبيق مبدأ الفحص المتتابع (cascade screening) وتم إيجاد عدة أفراد مصابين بهذا المرض في هذه العائلة. لقد تم إيجاد علاقة بارزة بين نتيجة sanger sequencing ومعدل مستوى LDL-C في الدم.

في الملخص, نستنتج أن هذا المرض قليل التشخيص والعلاج في مجتمعنا مما أدى الى زيادة الحالات المصابة بتصلب الشرايين التاجية المبكر وهذا من أحد أسباب زيادة الوفيات في مجتمعنا. عملية الفحص المتتابع للأمراض الجينية بشكل عام ولهذا المرض بشكل خاص هي عملية موفرة اقتصادياً وتؤدي للكشف عن حالات جديدة يتم علاجها مبكراً بالأدوية الخافضة للكولسترول وبالتالي التقليل من فرص الإصابة بالجلطات القلبية والوفاة المفاجئة والمبكرة لدى أفراد في عقدهم الثالث او الرابع. قد تكون الطفرة الجينية NM_000527.2: c.1210A>G p. (Thr404Ala) مسببة لمرض ارتفاع كولسترول الدم الوراثي بحيث أن استبدال A>G له علاقة بارزة مع معدل مستوى LDL-C في الدم.