ANALYSIS OF THE ASSOCIATION BETWEEN CETP *Taq1B* POLYMORPHISM (rs708272) AND CHOLESTEROL LOWERING EFFECTS AMONG STATIN USERS WITH HYPERLIPIDAEMIA FROM HUSM: A PILOT STUDY

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by

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LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

Abbreviation	Name
CETP	Cholesteryl ester transfer protein
ddH_2O	Deionized distilled water
DDI	Drug-drug interaction
dNTP	Deoxynucleotide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DM	Diabetes mellitus
EDTA	Ethylenediaminetetraacetic acid
gDNA	Genomic DNA
H	Hour
HDL	High density lipoprotein
HMGCoA	3-Hydroxy-3-methylglutaryl coenzyme A
HPL	Hyperlipidaemia
HPT	Hypertension
IHD	Ischemic heart disease
LDL	Low density lipoprotein
mg	Milligram
$MgCl_2$	Magnesium chloride

Abbreviation	Name
MTF	Metformin
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
s	Second
TAE	Tris-acetate-EDTA
TC	Total cholesterol
TG	Triglycerides
UV	Ultraviolet
VLDL	Very low density lipoprotein
%	Percentage
µl	Microliter

ANALYSIS OF THE ASSOCIATION BETWEEN CETP *Taq1B* POLYMORPHISM (rs708272) AND CHOLESTEROL LOWERING EFFECTS AMONG STATIN USERS WITH HYPERLIPIDAEMIA FROM HUSM: A PILOT STUDY

ABSTRACT

Statin, a cholesterol lowering agent, is widely used for treatment of hyperlipidemia (HPL) nowadays. Generally, statin is effective in reducing cholesterol level. However, there is a marked inter-patient variation with regard to response to statin treatment possibly due to differences in patients' genetic makeup, their demographic profiles, and other clinical factors. A single nucleotide polymorphism(SNP) in Cholesteryl Ester Transfer Protein (CETP) *Taq1B* (rs708272) gene was reported as a promising predictive genetic marker for cholesterol lowering effect in many populations Until now, there is no genetic association study among Malaysian population has been explored the influence of the particular SNP on statin's cholesterol lowering effects. Hence, we aim to study the association of CETP *Taq1B* (rs708272) variants together with other clinical factors on cholesterol lowering effect of statin among HPL patients from Hospital Universiti Sains Malaysia (HUSM), Kelantan. This is a retrospective study which involves 81 HPL patients. The details regarding demographic and clinical characteristics of patients were obtained via interview and patient's medical record. The genotyping of DNA samples of HPL patients were performed by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The results indicate that there were no significant association between patients' demographic profiles as well as clinical factors and their statin cholesterol lowering effects. Interestingly, variant in CETP *Taq1B* rs708272 has been associated with statin's cholesterol lowering effects for a certain degree. In homozygous

mutant patient with B2B2 (AA) genotype has higher HDL-C level ($P<0.05$) than other genotypes (1.39±0.33 vs 1.29±0.32 and 1.26±0.29). The LDL-C and TC level were decreased significantly ($P<0.01$) in heterozygous variant patient with B1B2 (AG) genotype. While TG level was decreased significantly in B1 carrier. This finding warrants further multicenter investigation to confirm the association of the SNP with regards to the statin effectiveness on both LDL-C and HDL-C profile.

ANALISIS PERKAITAN ANTARA POLIMORFIK DALAM GEN CETP *Taq1B* (rs708272) DAN KESAN PENURUNAN KOLESTEROL DALAM KALANGAN PESAKIT HYPERLIPIDAEMIA YANG MENGGUNAKAN STATIN DARI HUSM: KAJIAN PERINTIS

ABSTRAK

Statin adalah agen penurun kolesterol yang digunakan secara meluas untuk rawatan hiperlipidemia (HPL) pada masa kini. Secara amnya, statin berkesan dalam mengurangkan aras kolesterol. Walaubagaimanapun, terdapat variasi inter-pesakit yang ketara bagi tindak balas terhadap rawatan dengan statin kemungkinan disebabkan oleh beberapa faktor seperti faktor genetik, demografi dan klinikal. Polimorfisme nukleotida tunggal (SNP) dalam gen "Cholesteryl Transfer Protein" (CETP) *Taq1B* (rs708272) dilaporkan sebagai peramal genetik untuk mengurangkan aras kolesterol. Sehingga kini, tiada kajian perkaitan genetik bagi SNP yang dikaji berkaitan dengan keberkesanan statin dalam kalangan penduduk Malaysia. Oleh itu, tujuan kami ialah untuk mengkaji perkaitan antara CETP *Taq1B* (rs708272) varian bersama dengan faktor klinikal yang lain terhadap kesan penurunan aras kolesterol oleh statin dalam kalangan pesakit HPL dari Hospital Universiti Sains Malaysia (HUSM), Kelantan. Ini adalah kajian retrospektif yang melibatkan 81 pesakit HPL. Butiran mengenai ciri demografi dan ciri klinikal pesakit telah diperolehi melalui temuduga dan rekod perubatan pesakit. Genotip sampel DNA pesakit HPL dilakukan dengan menggunakan teknik "Polymerase Reaction-Restriction Fragment Length Polymorphism" (PCR-RFLP). Keputusan menunjukkan bahawa tiada perkaitan yang ketara antara profil demografi pesakit dan faktor klinikal terhadapkesan penurunan kolesterol dalam kalangan pengguna statin. Menariknya, varian dalam CETP *Taq1B* rs708272 telah menunjukkan perkaitan dengan kesan penurunan kolesterol dalam kalangan pengguna statin dalam beberapa tahap tertentu. Pesakit mutan homozigot iaitu genotip B2B2 (AA) mempunyai tahap HDL-C ($P<0.05$) yang lebih tinggi daripada genotip lain (1.39 ± 0.33

vs 1.29 ± 0.32 dan 1.26 ± 0.29). Tahap LDL-C dan TC menurun dengan ketara ($P<0.01$) dalam kalangan pesakit varian heterozigot iaitu genotip B1B2 (AG). Manakala, tahap TG berkurangan dengan ketara dalam pembawa B1. Penemuan ini memerlukan penyelidikan yang lebih lanjut untuk mengesahkan perkaitan antara SNP berkenaan dengan keberkesanan statin berdasarkan profil LDL-C dan HDL-C.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Hyperlipidaemia (HPL) is a high concentration of cholesterol or triglycerides in blood cause by several factors such as overweight, poor diet, inactive lifestyle, alcoholism, smoking, inherited genes and medical condition. This disease also associated with cardiovascular disease. In 2008, WHO revealed that the prevalence of raised cholesterol in Malaysia especially quite high which is 50-59.9% for both sexes. There was a study shown that by ethnic groups, the prevalence of HPL among Malays, Chinese, Indians and other races were 51.0%, 40.8%, 41.6% and 34.4% respectively (Lin et al., 2018). Ministry of Health has tried their best to promote health awareness and produce a better treatment to ensure that the percentage of HPL patient reduced. As far as we concerned, the early detection of HPL and with appropriate intervention is crucial to a good prognosis.

Therefore, several types of cholesterol lowering agents have been used to treat hyperlipidaemia patients, however statin has been the first-line treatment because of its established and affordability (Miziorko, 2011). Statins were structural analogues of HMG-coenzyme A reductase. They act by inhibiting the rate limiting enzyme (HMG-coenzyme A reductase) in the biosynthesis of cholesterol (mevalonate pathway) in the liver. By inhibiting this enzyme, statins significantly reduced plasma levels of total cholesterol (TC), LDL and ApoB. Meanwhile, statins also caused a modest decreased in plasma triglycerides (TG) and a small increased in plasma level of HDL (Eiland et al., 2010).

Although statin has been the first line treatment and proven efficacy in HPL patients, sometimes it does not apply to some patient. Interpatient variability in response to treatment may be associated with several factors such as age, gender, comorbidities, concomitant drug, supplementation and genetic variation. Several studies have demonstrated that some of this factor can influence cholesterol lowering effect. For example, a previous study related to Cholesteryl Ester Transfer Protein (CETP) *Taq1B* polymorphism showed that B2 allele has high HDL than B1B1 in atherosclerosis and protective effect such as increased HDL level was more significant in men than women and in non-smoker than a smoker (Rejeb et al., 2008).

1.2 The Significant of the Study

This study is relevant to be conducted in order to get a data regarding the prevalence and the factors that can affect statin effectiveness in HPL patients in our population. Until now, there is limited data and evidence describing this relationship in our population (Malaysian).

Besides that, genetic variation encoded within lipid metabolism is the most prominent genetic factors associated with cholesterol lowering effect. However, due to discrepancy of findings among previous studies, no accurate genetic predictive markers for statin effectiveness have been identified so far. Hence the current study intended to investigate the impact of selected single nucleotide polymorphism (SNP) in gene encode in lipid metabolic pathway in cholesterol lowering effect. Genetic polymorphism in Cholesteryl Ester Transfer Protein (CETP) *Taq1B* is the potential genetic marker that associated with statin's cholesterol lowering effect and of interest for this study (Li et al., 2014; Ahmed et al., 2011).

1.3 Objectives of the Study

1.3.1 General Objective

To analyse the association between CETP *Taq1B* polymorphism (rs708272) and cholesterol lowering effect among statin users with hyperlipidaemia from HUSM.

1.3.2 Specific Objectives

1) To perform SNP genotyping for CETP *Taq1B* gene (rs708272) by using Restriction Fragment Length Polymorphism (RFLP) method.

2) To determine the association of CETP *Taq1B* polymorphism and cholesterol lowering effect among statin users with HPL.

3) To determine the extent by which factors (demographic factors, clinical factors and genetic variant) contribute to the cholesterol lowering effect among HPL patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Hyperlipidaemia

2.1.1 Introduction to Hyperlipidaemia

Hyperlipidaemia is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. It is also called hypercholesterolemia/ hyperlipoproteinemia (Amit et al., 2011). Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, hyperlipidaemia can also be described as an elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL).

Hyperlipidaemia is divided into two subtypes such as primary hyperlipidaemia and secondary hyperlipidaemia. Primary hyperlipidaemia usually take place as a result of genetic problems i.e., mutation within receptor protein, which may be due to single (monogonic) gene defect or multiple (polygenic) gene defect. This type may occur as a result of change in dietary and lack of proper physical activities. Secondary hyperlipidaemia arises as a result of other underlining diseases like diabetes, myxoedema, nephritic syndrome, chronic alcoholism, with use of drugs like corticosteroids, oral contraceptives and Beta blockers (Joseph, 2005).

Human body is complex machine for maintaining the homeostasis of various organ and organ system. Any undesirable change will disturb the balance resulting in diseased state. Lipids are fats in the blood stream, commonly divided into cholesterol and triglycerides. Cholesterol circulates in the bloodstream and is involved in the structure and function of cells. Triglycerides (TG) are best viewed as energy that is either used immediately or stored in fat cells. TG is manufactured in the liver from the foods or by being absorbed from the intestine (Ankur et al., 2012). Arteries are normally smooth and unobstructed on the inside, but in case of increased lipid level, a sticky substance called plaque is formed inside the walls of arteries. This leads to reduced blood flow, leading to stiffening and narrowing of the arteries. It has been proved that elevated plasma levels of cholesterol and of LDL are responsible for atherosclerosis in man, and epidemiological data suggests that elevated plasma levels of HDL have a protective effect (Grundy and Vega, 1998). Hence, it is important to maintain lipid level to reduce the risk of heart disease. To maintain this lipid level, one should has LDL less than 130 mg/dL or < 70 if he/she has established diagnosis of diabetes, HDL greater than 40 mg/dL (men) or 50 mg/dL (women), Total cholesterol less than 200 mg/dL and Triglycerides less than 200 mg/dL or 150 if he/she has established heart disease or diabetes (Barbara et al., 2005).

2.1.2 Epidemiology of Hyperlipidaemia

According to WHO Global estimates, hyperlipidaemia caused one third of ischemic heart disease and one fifth of global cerebrovascular disease and equated to nearly 2.6 million deaths every year worldwide (WHO, 2014). Previous studied showed that various lipid abnormalities such as increased total and low- density lipoprotein cholesterol, low concentration of high-density lipoprotein cholesterol and high triglycerides concentrations and their combinations have been implicated as potential independent predictors of CVD (Supiyev et al, 2017). Cardiovascular disease (CVD) has been reported as a leading cause of death among adults in the United State and people with hyperlipidaemia was at roughly twice the risk of developing CVD as compared to those with normal total cholesterol level (Samantha, 2017).

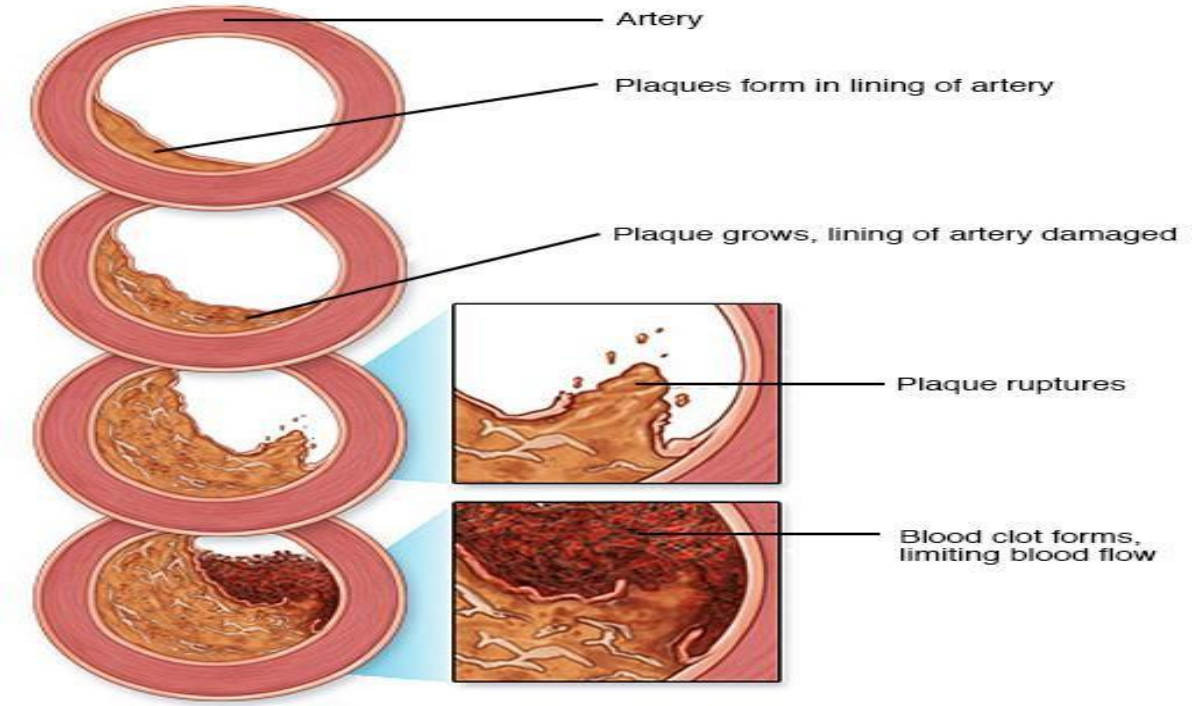

Figure 2.1: Hyperlipidaemia (Mayo Clinic, 2017)

This health problem was a common illness in Asia especially in Malaysia. This kind of disease caused by overweight, poor diet, inactive lifestyle, alcoholic, smoking, inherited genes and medical condition (Kathleen, 2017).

The raised of total cholesterol was a major cause of disease burden in both the developed and developing world as a risk factor for ischemic heart disease and stroke. In 2008 the global prevalence of raised total cholesterol among adults (≥ 5.0 mmol/l) was 39% (37% for males and 40% for females). As per recorded, the mean total cholesterol changed little between 1980 and 2008 which falling by less than 0.1 mmol/L per decade in men and women globally (WHO, 2011).

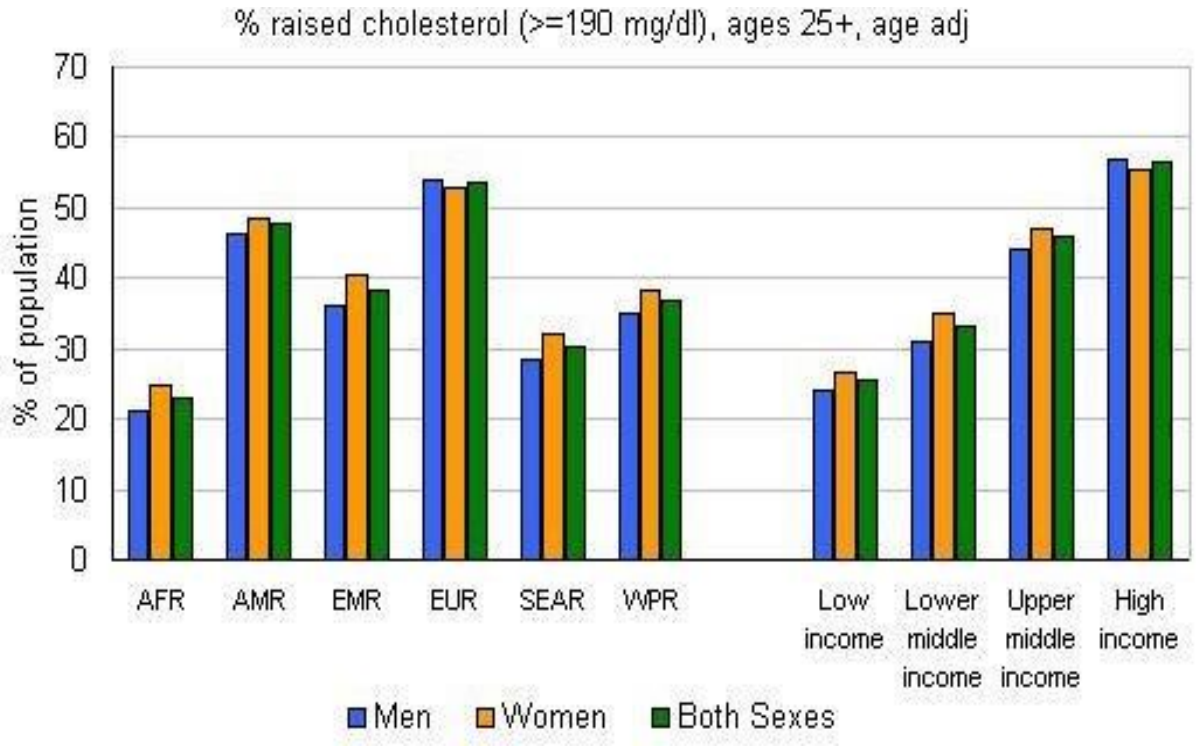

Figure 2.2: Prevalence of Raised Total Cholesterol worldwide (WHO, 2011)

In 2008, the prevalence of raised blood cholesterol among standardized 25+ aged of both sexes figure was produced by WHO which shown Europe (54%) as the highest, followed by the Americas (48%). The lowest were conquered by African Region (22.6 %) and South East Asian Region (29.0%) as stated in the figure 2.3.

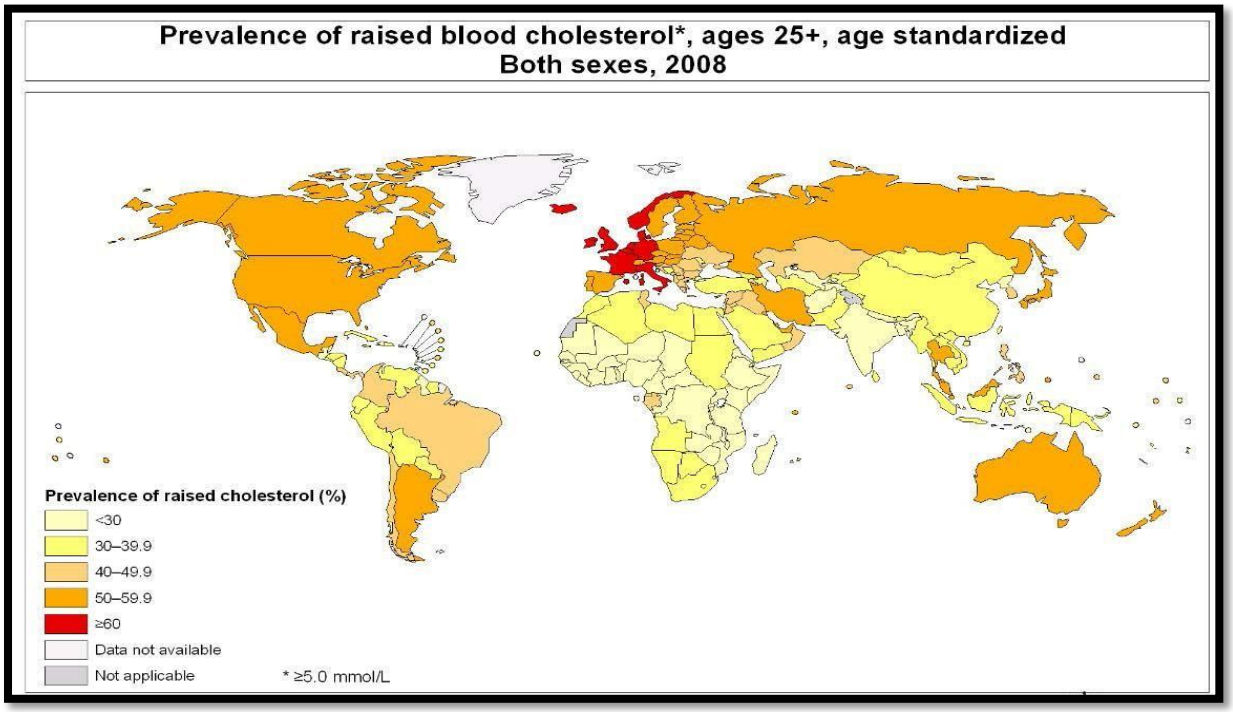

Figure 2.3: Prevalence of raised blood cholesterol with standardized age of both sexes (WHO, 2011)

Based on the figure 2.3, the prevalence of raised cholesterol (%) in Malaysia was around 50-59.9% which was quite high and our Ministry of Health has tried their best to promote health awareness and produce a better treatment. Malaysia consisted of multi-ethnic nation with three major ethnic groups such as Malays (63.1%), Chinese (24.6%) and Indians (7.3%). There was a study shown that by ethnic groups, the prevalence of hypercholesterolemia among Malays, Chinese, Indians and other races were 51.0%, 40.8%, 41.6% and 34.4% respectively (Lin et al., 2018).

2.1.3 Pathogenesis of Hyperlipidaemia (HPL)

Fats played a vital role in body's metabolic process but if it was high, it increased the risk of coronary heart disease (CHD). There were two common types of lipid abnormalities either high blood cholesterol levels (hypercholesterolemia) or high blood triglycerides levels (hypertriglyceridemia) (Simeon, 2011).

During lipid metabolism (Figure 2.4), lipoprotein helped cholesterol moved around in blood stream. Lipoprotein contained a nonpolar core in which molecules of hydrophobic lipid were packed to form an oil droplet. The core consists of triglycerides and cholesteryl esters in varying proportions. There are five major classes of lipoproteins including chylomicrons, very-low-density lipoprotein (VLDL), remnants, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). A polar surface coat of phospholipid surrounded the core. The coat stabilized the lipoprotein particle so that it can remain as solution in plasma. Each lipoprotein particle contained specific proteins, apoproteins that were particularly exposed at the surface. The apoprotein binds to specific enzymes or transport proteins on cell membranes, thus directing the lipoprotein to its sites of metabolism. Almost all the dietary fats were absorbed from the intestinal lumen into the intestinal lymph and packed into chylomicrons (lipoprotein). These lipoproteins move into the blood stream where triglycerides hydrolyzed into glycerol and nonesterified fatty acids by endothelial lipoprotein lipase. After which the chylomicron remnants were absorbed in the liver and packaged with cholesterol, cholesteryl esters and ApoB100 to form VLDL. After released of VLDL into blood stream, it was converted into IDL by lipoprotein lipase and hepatic lipase, so phospholipids and apolipoproteins transferred back to HDL. Furthermore, after the hydrolysis by hepatic lipase, IDL was converted to LDL and loss more apolipoproteins (McLaren et al., 2011).

Peripheral cholesterol returned to the liver by reverse cholesterol transport pathway using HDLs which were originally synthesized by the liver and released into the blood. In the blood, HDL cholesterol was esterified by Lecithin cholesterol acyltransferase (LCAT) to cholesteryl ester and transferred to VLDL (lipoprotein) and chylomicrons (lipoprotein) returned to the liver via LDL receptor. Cholesteryl ester was transferred to LDL particles by CETP and then subjected to LDL-receptors mediated endocytosis. Finally, cholesteryl esters were hydrolyzed to cholesterol and extracted from the body as bile acid. In this way a cycle has been established in which LDLs deliver cholesterol to extrahepatic tissue and cholesterol was then returned from extrahepatic tissue via HDLs. Cholesterol in the blood originates partly from food and partly from de novo synthesis of cholesterol. Cholesterol was manufactured primarily in the liver and then carried in the bloodstream by low density lipoprotein (LDL). Cholesterol and other fats do not dissolve in water so cannot travel through the blood unaided. Therefore, lipoproteins were formed in the liver to transport cholesterol and other fats through the bloodstream. Cholesterol was returned to the liver from other body cells by high density lipoprotein (HDL). From there, cholesterol was secreted into the bile, either unchanged or after conversion to bile acids (Hegele et al., 2009).

Basically, cholesterol was essential for the formation of cell membranes and the manufacture of several hormones, but it was not required from the diet because the liver produces all the cholesterol the body needs. If blood cholesterol levels were elevated, large amounts of LDL (bad) cholesterol can deposited in the arterial walls. These represented the first stage in the narrowing of arteries (atherosclerosis). Generally, hypercholesterolemia causes no symptoms, so preventive measures and regular measurement of cholesterol level was important for people in high-risk categories. It was dangerous especially when HDL (good) cholesterol levels were low. Left untreated, hypercholesterolemia can eventually lead to a heart attack due to CHD or a stroke due to narrowed arteries supplying the brain (Simeon, 2011).

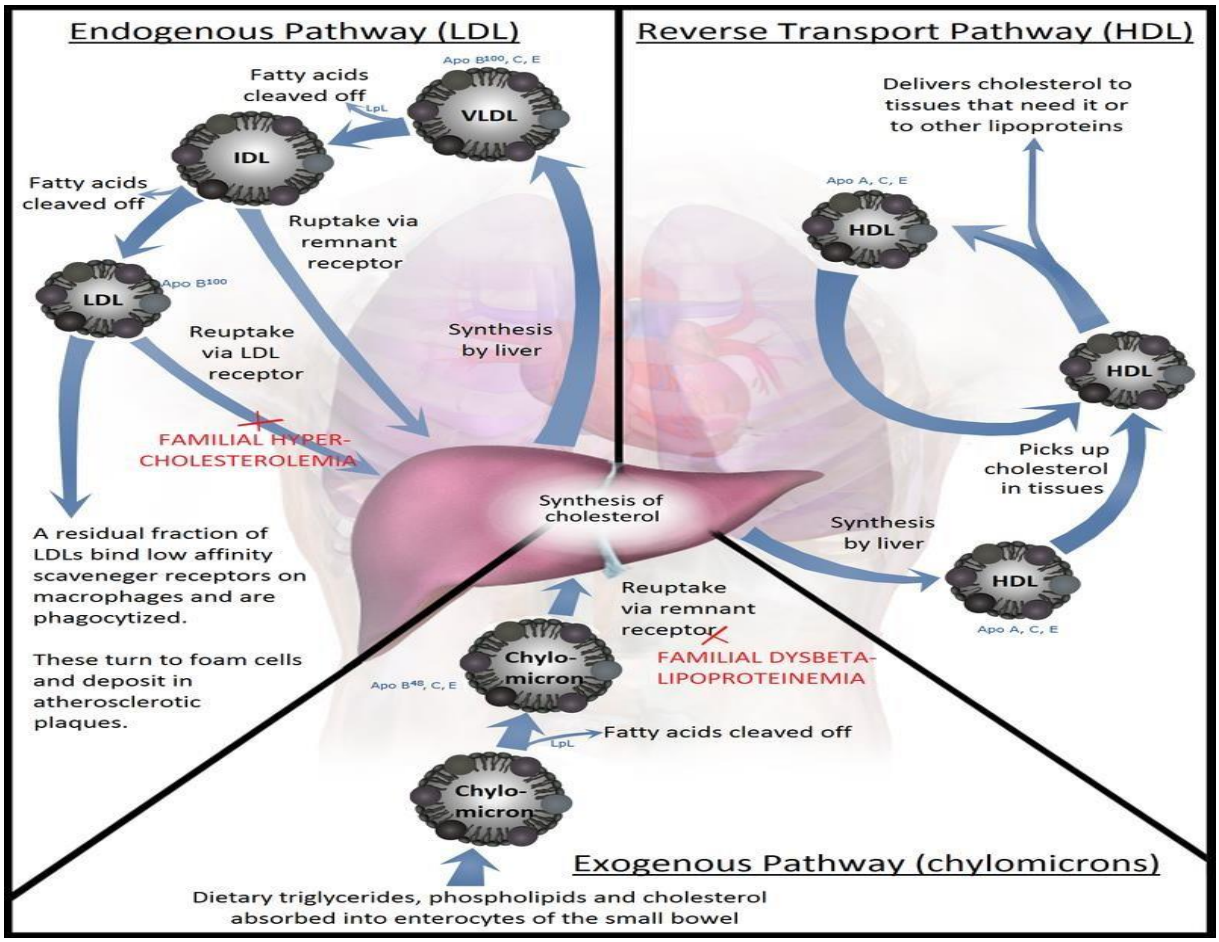

Figure 2.4: Lipid metabolism (Wikipedia)

2.1.4 Risk Factors of Hyperlipidaemia (HPL)

Cholesterol is a wax, fat-like substance made in liver and found in certain foods from animals such as dairy products, eggs, and meat. Blood cholesterol level was not only affected by food but also by how quickly body makes LDL (bad) cholesterol and disposed of it. Human body needed cholesterol to function properly but too much of cholesterol increased the risk of heart disease. In fact, body makes all the cholesterol it needs and it was not necessary to have any additional cholesterol from food. There were several factors that contributed to high cholesterol as below.

Diet

The Trans fats (found in some commercially baked cookies and crackers), saturated fat (mostly in food that came from animal), sugar, and (to a lesser extent) cholesterol which came only from animal products in the food can raise total and LDL cholesterol levels (Suzanne, 2018). Poor diet i.e. with a fat intake greater than 40 % of total calories, saturated fat intake greater than 10 % of total calories; and cholesterol intake greater than 300 milligrams per day increased the risk of HPL (Durrington, 1995). Therefore, reduced the amount of saturated fat and cholesterol in food was a very important step in reducing blood cholesterol levels.

Weight

Being overweight can make LDL cholesterol level go up and HDL level go down. By losing weight may help to lower it. Weight loss also helped to lower triglycerides and raised HDL ("good") cholesterol levels. Exercise helps boost body's HDL cholesterol while increased the size of the particles that make up LDL cholesterol, which makes it less harmful (Suzanne, 2018).

Physical activity/exercise

Regular physical activity helped to lower LDL cholesterol (the "bad" cholesterol) and raised HDL cholesterol (the "good" cholesterol) levels. It also helps to lose weight (Suzanne, 2018).

Age and Gender

After menopause, a woman's LDL cholesterol level ("bad" cholesterol) goes up, as well as her risk for heart disease. The risk increased as women and men got older. Men aged ≥ 45 years old and women aged ≥ 55 years old were at increased risk of high cholesterol and heart disease (Suzanne, 2018).

Family history and Heredity

The risk of high cholesterol increased if a father or brother was affected by early heart disease (before age 55) or a mother or sister was affected by early heart disease (before age 65) (Suzanne, 2018). Heredity has also been a modifying factor for the progression of hyperlipidaemia as it has been noted that the genes partly determine the amount of cholesterol body makes (Durrington, 1995).

Alcohol

Alcohol intake increased HDL ("good") cholesterol but did not lower LDL ("bad") cholesterol. However, drinking too much alcohol can damage the liver and heart muscle, lead to high blood pressure, and raised triglycerides. Because of the risks, alcoholic beverages should not be used as a way to prevent heart disease (Durrington, 1995).

Smoking

Cigarette smoking damaged the walls of blood vessels, making them likely to accumulate fatty deposits. Smoking also lowered level of HDL cholesterol (Durrington, 1995).

Other disease

It has been noted that kidney disease, metabolic syndrome, nephrotic syndrome, underactive thyroid gland, polycystic ovarian syndrome can predispose to hyperlipidaemia (Durrington, 1995).

2.2 Lipid Metabolism

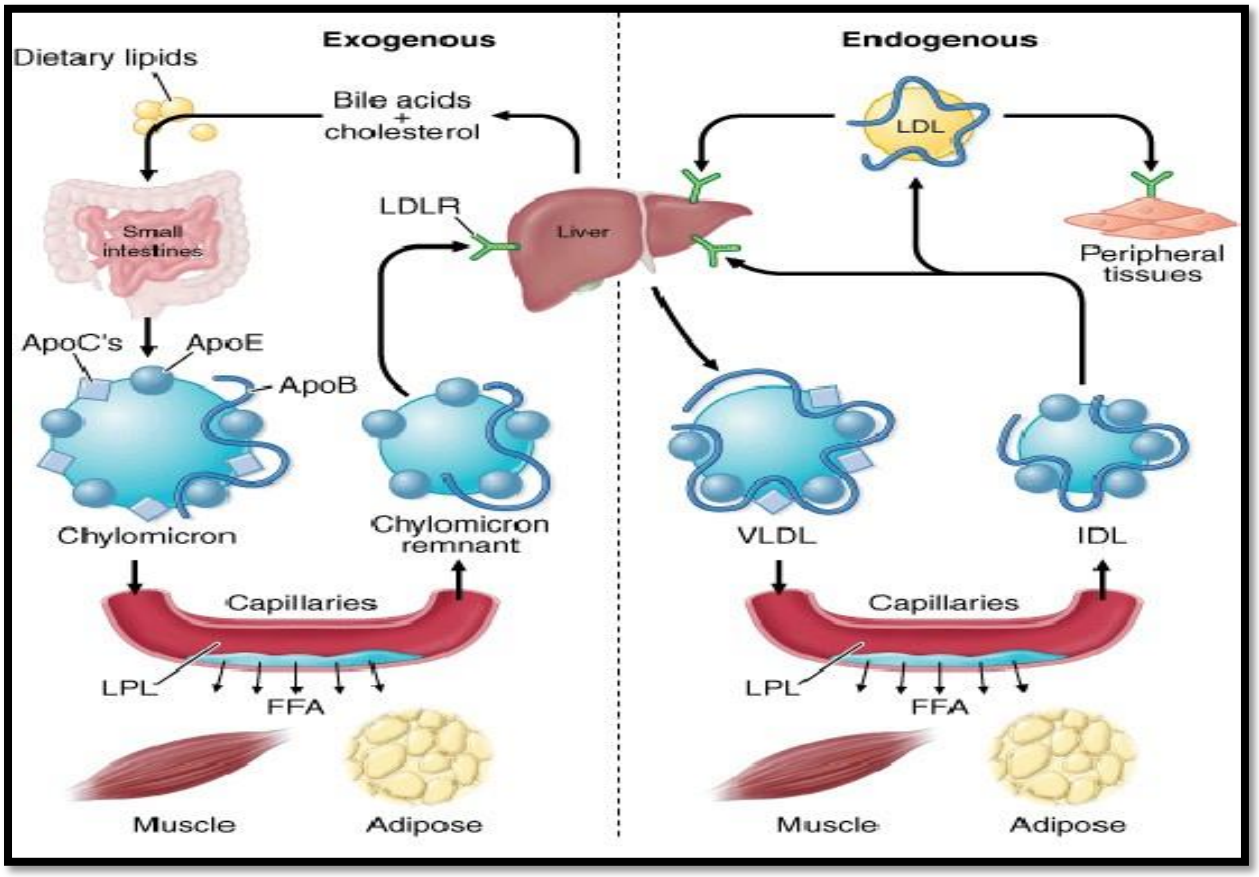

Figure 2.5: Schematic of the exogenous and endogenous lipid metabolism pathways (Catherine, 2014)**.**

Cholesterol plays a vital role in several diseases such as coronary heart disease, stroke, diabetes mellitus, hypertension, hyperlipidaemia, obesity and pancreatitis. Therefore, a look into lipid metabolism is important to understand how cholesterol has been process in our body. In lipid metabolism pathway, circulating cholesterol derived from either the endogenous or exogenous pathway (figure 2.5). All cholesterol will be packaged into lipoprotein particles as part of their metabolism pathway and covered with a specific complement of apolipoproteins (Catherine, 2014).

As part of the endogenous pathway, the liver is responsible for the packaging of very low-density lipoprotein (VLDL) particles which are hydrolyses to intermediate density lipoprotein (IDL), returned to the liver so that they may be repackaged as low-density lipoprotein (LDL) then taken from the circulation by peripheral tissues. The exogenous pathway is necessary for the packaging and secretion of chylomicrons and their remnants utilizing both the small intestine and the liver. Hydrophobic lipids cholesterol and triglycerides (TG) are packaged into lipoprotein particles, which are coated with apolipoproteins that enable their solubility and transport within the circulation. ApoB100 is essential for packaging and secretion of VLDL particles from the liver. These surface proteins are also vital for lipoprotein transport in the circulation as the otherwise hydrophobic lipids would be insoluble in plasma. Lastly and importantly, apolipoproteins are used for lipoprotein particle recognition by their receptors, which is essential for their uptake into peripheral cells and removal from the circulation (Fauci et al. 2008).

2.2.1 Low Density Lipoprotein (LDL)

The endogenous pathway, which is responsible for the majority of cholesterol in circulation, requires the de novo synthesis of cholesterol by the liver, resulting in secretion of VLDL particles (Russell, 1992). The rate limiting enzyme involved in this process is 3-hydroxy-3-methyl-glutaryl-CoA (HMG Co-A) reductase, the key target of statin drugs which are used for cholesterol lowering. VLDL particles consisting of fatty acids, free cholesterol and TAG are packaged by and coated with the apolipoprotein apoB100, which is secreted from hepatocytes in the liver.

Like chylomicrons, circulating VLDL is acted upon by lipoprotein lipase resulting in VLDL remnants termed IDL particles. IDL then returns to the liver where it is hydrolyzed by hepatic lipase resulting in particles referred to as LDL. The major apoprotein of LDL remains the same: apoB100. The apoB-100 surface protein of LDL is recognized by tissue cells by the LDL receptor (LDLr), which is expressed on the surface of cells required cholesterol for membrane building. Excess cellular cholesterol may then also be subjected to the reverse cholesterol transport (RCT) pathway mediated by the ATP-binding cassette A-1 transporter (ABCA1), which enables the uptake of cholesterol by apo-AI, the main surface protein of HDL particles (Oram et al., 2001).

2.2.2 Triglycerides (TG)

The exogenous pathway refers to the absorption of dietary lipids by intestinal epithelial cells. Ingested lipids are packaged into chylomicron particles, which consist mainly of triglycerides, phospholipids and cholesterol, and are coated in the protein apolipoprotein B-48. The major role of chylomicrons is to transfer energy, in the form of fatty acids, to peripheral cells. This is mediated by the hydrolysis of triglycerides contained in circulating chylomicrons by lipoprotein lipase. The resulting chylomicron remnant particles are then reabsorbed by the liver and the cholesterol content is either used to generate new lipoprotein particles or excreted through the bile duct (Ramasamy, 2013).

2.2.3 High Density Lipoprotein (HDL)

Excess cellular cholesterol may then also be subjected to the reverse cholesterol transport (RCT) pathway mediated by the ATP-binding cassette A-1 transporter (ABCA1), which enables the uptake of cholesterol by apo-AI, the main surface protein of HDL particles (Oram, 2001). HDL recognized by HDL receptors expressed by the liver are taken up and the cholesterol is recycled to be reintroduced to new VLDL particles, or excreted by means of the bile duct (Russell, 1992).

2.3 Treatment for Hyperlipidaemia (HPL)

2.3.1 Overview

Currently, anti-hyperlipidaemia drugs contained five major classes (Figure 2.6) that include statins, fibric acid derivatives, bile acid binding resins, nicotinic acid derivatives and drugs that inhibit cholesterol absorption (Joseph, 2011). The most common drug prescribed in Malaysia was statin or ezetimibe and sometimes a combination of both drugs. Monotherapy has been shown to be effective in treating hyperlipidaemia, but combination therapy may be required for a comprehensive approach (MOH, 2011).

2.3.2 3-Hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase Inhibitors (Statins).

Statins are indicated for the prevention of cardiovascular disease and are among the most prescribed classes of medication (MOH, 2011). Their inhibition of HMGCR results in decreased intrahepatic cholesterol synthesis, upregulation of hepatocyte surface LDL-C receptors, increased LDL-C uptake by hepatocytes, and ultimately decreased systemic concentration of LDL-C (Kitzmiller et. al, 2016). There are several class of statin such as Lovastatin, Simvastatin, Pravastatin, Fluvastatin, Atorvastatin and Rosuvastatin. Statins were broadly prescribed in the treatment of hypercholesterolemia and have been linked to the reduced incidence of coronary morbidity and mortality in high-risk adults (Belay et al., 2006).

2.3.3 Selective cholesterol absorption inhibitor (Ezetimibe)

Ezetimibe was another LDL-lowering drug that blocked the absorption of cholesterol by the small intestine. Ezetimibe appeared to be safe and mostly used in patients with statin intolerance. Besides that, a combination with statin reduced cardiovascular events in patients with chronic kidney disease (Baigent et al. 2011).

2.3.4 Fibric acid derivatives (Fibrates)

Fibrates were primarily triglyceride-lowering agents that also lower VLDL-C. It was used for treatment of severe hypertriglyceridemia to prevent development of acute pancreatitis. Like ezetimibe, it also an alternative in people who cannot tolerate statins. The combination of a statin and a fibrate was attractive for mixed hyperlipidaemia (Shattat, 2014).

2.3.5 Nicotinic acid derivatives (Niacin)

Niacin effectively lowered triglycerides, moderately raised HDL-C and moderately reduced LDL-C. It was a water-soluble vitamin of type B and the oldest lipid lowering agent used to treat hyperlipidaemia and proved to decrease cardiovascular morbidity and total mortality. In the previous study shown that niacin combined with a statin reduced subclinical atherosclerosis. On the other hand, for patients with statin intolerance, the combination of niacin and ezetimibe can effectively lower LDL-C levels (Jelesoff et al., 2006).

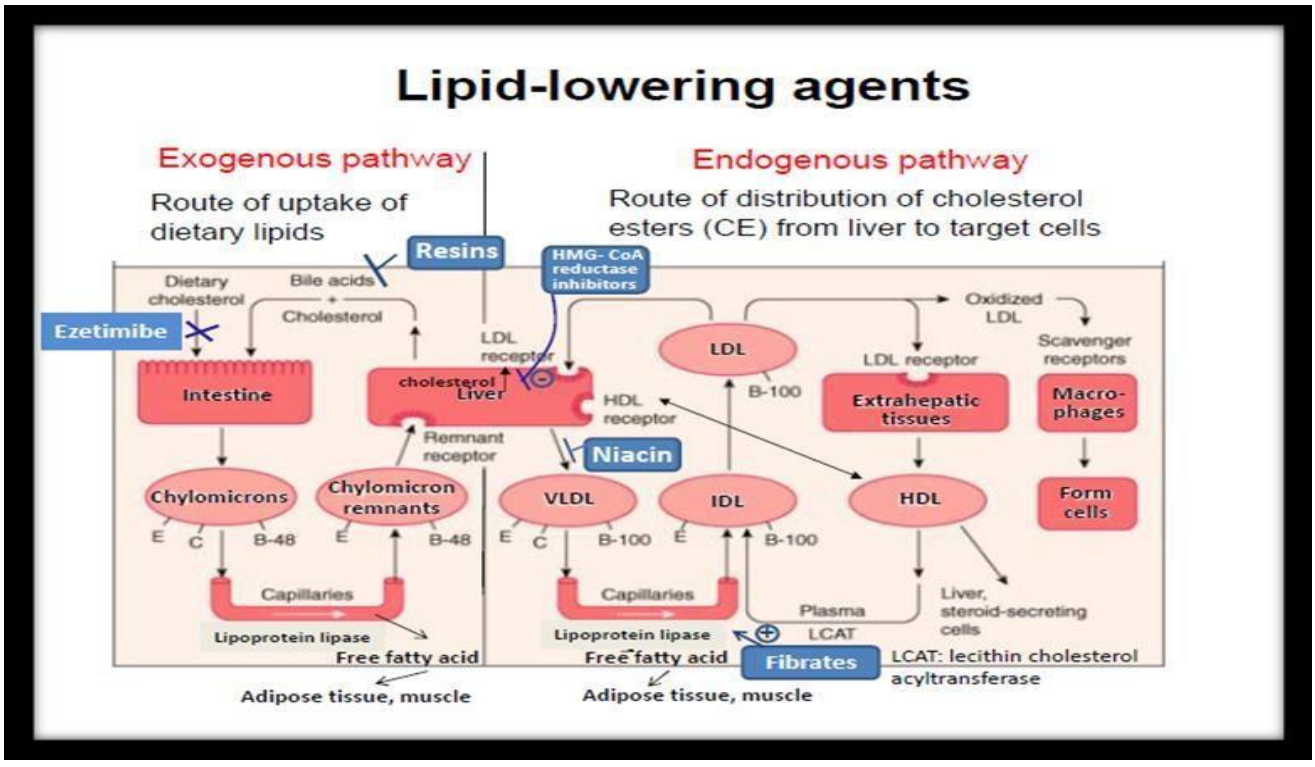

Figure 2.6: Lipid lowering agents (Isam, 2016)

2.4 Statin as First Therapy for Hyperlipidaemia

Statins are powerful LDL lowering drugs. They blocked cholesterol synthesis in the liver and raised LDL receptors, which removed LDL from the blood stream (Figure 2.7). Statins also lowered VLDL, the other atherogenic lipoprotein. These agents reduce LDL-C by 25-55%. Statins have proven to be safe for most patients (Pasternak et al., 2002). They did not cause liver disease, cataracts, or hemorrhagic stroke. Rare patients experienced muscle damage characterized by marked elevations of creatine kinase, rhabdomyolysis, hemoglobinuria and acute renal failure. This was most likely to occur in who have complex medical problems (concurrent disease) and/or who were taking multiple medications (concomitant drugs). Recently statins have been linked to new onset diabetes (Preiss et al., 2011). Most cases of diabetes occur in patients who already have borderline diabetes. Occasional patients complain of cognitive dysfunction while taking statins. The possibility of these side effects indicated that statin therapy must balance benefit versus risk. Fortunately, the risk for serious side effects was low.

2.4.1 Mechanism of Action of Statin

Most of the cholesterol that courses through the circulation does not enter the system directly from dietary sources. Rather, it is synthesized in the smooth endoplasmic reticulum by means of a series of chemical reactions that at one point are catalyzed by HMG CoA reductase. The first way to block cholesterol synthesis is by interrupting the conversion of HMG CoA to mevalonate (so that mevalonate cannot generate cholesterol). In order for HMG CoA to become mevalonate, the reaction must be catalyzed by the enzyme HMG CoA reductase. If this enzyme is blocked, mevalonate cannot be generated and cholesterol cannot be synthesized.

This is the principal mechanism of action of the most popular and most effective of the "cholesterol-blocking" medicines, the HMG CoA reductase inhibitors, which are collectively known as "statins". By inhibiting this enzyme (HMG CoA reductase), statins significantly reduced plasma levels of total cholesterol (TC), LDL and ApoB. Meanwhile, statins also caused a modest decreased in plasma triglycerides and a small increased in plasma level of HDL (Eiland et al., 2010).

Since statin increase HDL level and decrease triglycerides level, Cholesteryl Ester Transfer Protein (CETP) also contribute to both of this cholesterol. CETP is involved in reverse cholesterol transport from peripheral tissues. This reverse transport pathway is responsible for HDL level. CETP *TaqIB* genotype seems to be associated with modified HDL response; individuals with the B1B1 genotype have been reported to display the greatest HDL increases in response to statins (Zineh, 2007).

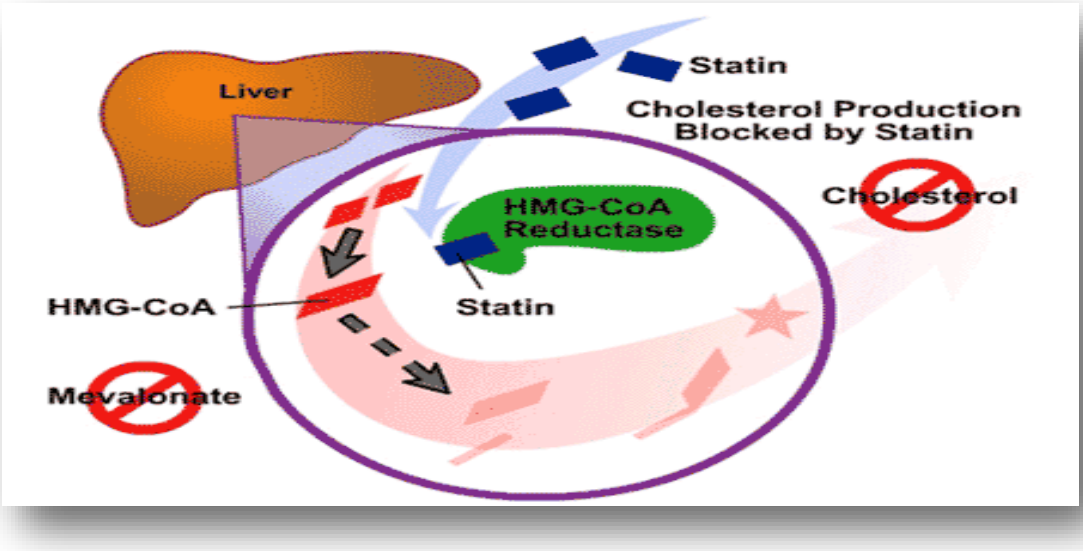

Figure 2.7: Statin-Mechanism of Action (Medscape)

2.5 Cholesteryl Ester Transfer Protein (CETP)

2.5.1 Overview of CETP

Cholesteryl ester transfer protein (CETP) gene is also called as a plasma lipid transfer protein, was located on the 16th chromosome. Based on figure 2.8, CETP is a hydrophobic plasma glycoprotein that secreted mainly from the liver and circulates in plasma, bound mainly to HDL. It facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins by collected triglycerides from a very low density (VLDL) or low density lipoprotein (LDL) and exchanged them for cholesteryl esters from high density lipoprotein (HDL) and vice versa. A CETP deficiency is linked to increased HDL levels and decreased LDL levels (Durrington, 2012).

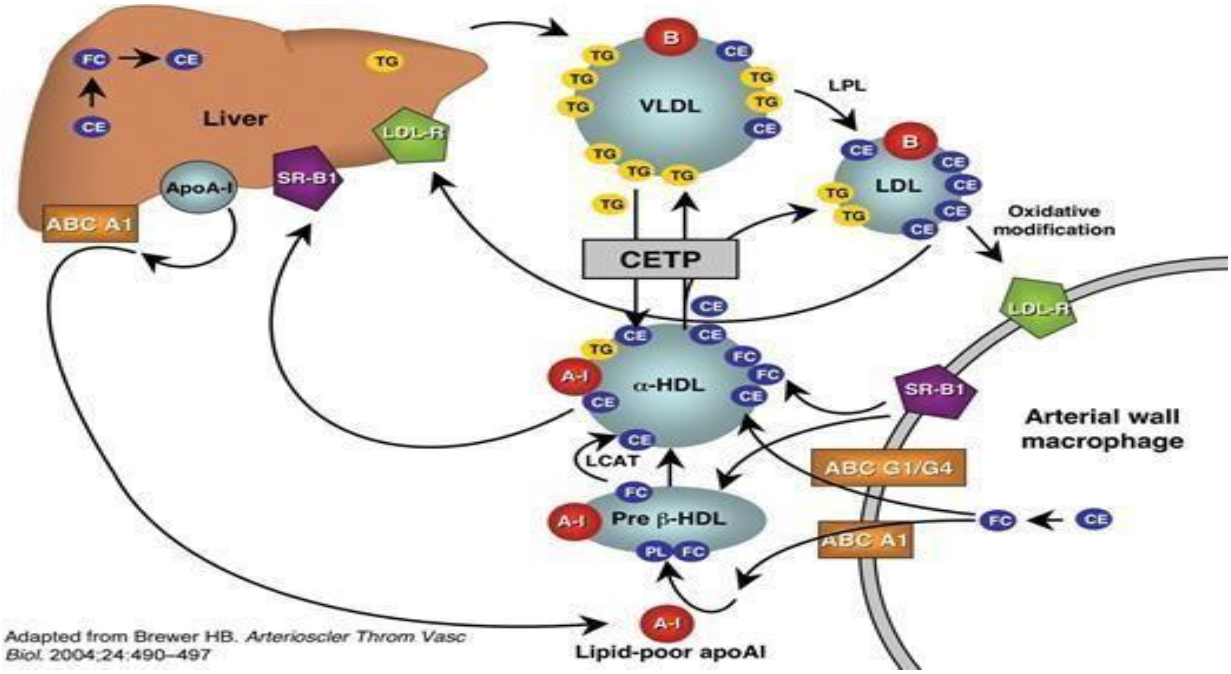

Figure 2.8: CETP Pathway (Shah, 2007)