ASSOCIATION OF GENETIC POLYMORPHISMS AND THEIR ATTRIBUTING FACTORS WITH LIPID PROFILES AMONG OUTPATIENT STATIN USERS IN HOSPITAL USM

AMIRUL FAEZ BIN SHAMSUDIN

UNIVERSITI SAINS MALAYSIA

2022

ASSOCIATION OF GENETIC POLYMORPHISMS AND THEIR ATTRIBUTING FACTORS WITH LIPID PROFILES AMONG OUTPATIENT STATIN USERS IN HOSPITAL USM

by

AMIRUL FAEZ BIN SHAMSUDIN

Thesis submitted in fulfilment of the requirements

for the degree of

Master of Science

July 2022

ACKNOWLEDGEMENT

Alhamdulillah, praise to Allah SWT that I am able to finish the thesis. I would like to thank my supervisor, Dr Nur Salwani Binti Bakar, for her continuous guidance throughout my study, especially during my presentations, thesis and article writing. I would also like to express my gratitude to my co-supervisor, Assoc. Prof Dr Sarina Binti Sulong, who is generous enough to allow me to conduct laboratory procedures in Human Genome Center; Dr Imran Bin Ahmad, who provided his clinical expertise; and Dr Wan Nor Arifin Bin Wan Mansor, for assisting me in the statistical analysis part. Thank you to all my friends for helping me out whenever I needed help. I met many kind-hearted people during this period of my postgraduate study that taught me a thing or two, be it for my research or my life. Special gratitude to my parents, Shamsudin Bin Wagiman and Khalijah Binti Mohd Razi, for their encouraging words and my beloved family for always believing me in all the choices that I made. I could not finish the thesis without all of you. For that, I wish Allah SWT to bestow His kindness on all of you. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

~	Approximately
^{o}C	Degree celcius
α	Significance level
µL	Microliter
µM	Micromolar
P	P-value
bp	Base pair
g	Gram
g/L	Gram per liter
kDa	Kilodalton
kg/m^2	Kilogram per meter square
mg/dL	Miligram per deciliter
mg/day	Miligram per day
mL	Mililiter
mM	Milimolar
mmol/L	Milimol per liter
n	Number of samples
pH	pH value
s	seconds
U	Unit
U/L	Unit per liter
V	Volt
V/cm	Volt per centimeter

LIST OF ABBREVIATIONS

4S	Scandinavian Simvastatin Survival Study
ABC	ATP-binding cassette
ABCA1	ATP-binding cassette subfamily A member 1
ABCC2	ATP-binding cassette subfamily C member 2
ABCG2	ATP-binding cassette subfamily G member 2
ADP	Adenosine diphosphate
AGAT	Arginine: glycine amidinotransferase
AMPK	Adenosine monophosphate kinase
APOA1	Apolipoprotein-AI
APOAII	Apolipoprotein-AII
APOA5	Apolipoprotein-A5
APOB-100	Apolipoprotein-B100
APOE	Apolipoprotein-E
ASCOT-LLA	Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm
ASCVD	Arteriosclerotic cardiovascular diseases
ASIA	Atorvastatin and Simvastatin In Asia
AUC	Area under curve
BCRP	Breast cancer resistance protein
BMI	Body mass index
CARE	Cholesterol and Recurrent Events
CETP	Cholesteryl Ester Transfer Protein
CHD	Coronary heart diseases
CK	Creatinine kinase

COQ2	Coenzyme Q2
COQ10	Coenzyme Q10
CTT	Cholesterol Treatment Trialists
CYP2C9	Cytochrome P450 2C9
CYP3A4	Cytochrome P450 3A4
CYP450	Cytochrome P450
CVD	Cardiovascular diseases
ddH_2O	Double distilled water
deQTL	Differential expression quantitative trait loci
DILI	Drug-induced liver injury
DISCOVERY	Direct Statin Comparison of LDL-c values: an Evaluation of Rosuvastatin Therapy
DMSO	Dimethylsulfoxide
DNP	Double nucleotide polymorphisms
EAS	European Atherosclerosis Society
EEO	Electroendosmosis
ERα	Estrogen receptor α
ESC	European Society of Cardiology
EtBr	Ethidium Bromide
FDA	Food And Drug Administration
FH	Familial hypercholesterolaemia
FLP	Fasting lipid profiles
FRET	Fluorescence resonant energy transfer
GAA	Guanidinoacetic acid

GAMT	Guanidinoacetate methyl transferase
GATM	Glycine amidinotransferase
gDNA	Genomic DNA
GWAS	Genome-wide association study
HCL	Hypercholesterolaemia
HDL-c	High density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HPL	Hyperlipidaemia
HPT	Hypertension
HTG	Hypertriglyceridaemia
HUSM	Hospital Universiti Sains Malaysia
IDL	Intermediate density lipoprotein
IHD	Ischemic heart diseases
JUPITER	Justification for the Use of Statin in Prevention
KASP	Competitive allele-specific PCR
KRK	Klinik Rawatan Keluarga
LDL-c	Low density lipoprotein
LDLR	Low density lipoprotein receptor
LIPID MAPS	Lipid Metabolites and Pathway Strategy
LPL	Lipoprotein lipase
LXR	Liver X receptor
LRP	Low-density lipoprotein related receptor
MAF	Minor allele frequency
MI	Myocardial infarction

MNP	Multi-nucleotide polymorphisms
MRP	Multidrug resistance protein
MTP	Microsomal triglyceride transfer protein
NCEP ATP	National Cholesterol Education Program, Adult Treatment Panel
NHMS	National Health and Morbidity Survey
NPC1L1	Niemann-Pick C1-Like 1
OATP	Organic anion transporting polypeptide
OR	Odds ratio
PAPAGO-T	Pitavastatin and Atorvastatin Double-Blind Randomized Comparative Study Among High-Risk Patients, Including Those with Type 2 Diabetes Mellitus
PCSK9	Proprotein convertase subtilisin/kexin type 9
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphisms
PP	Pyrophosphate
RCT	Reverse cholesterol transport
RE	Restriction endonuclease
REVERSAL	Reversal of Atherosclerosis with Aggressive Lipid-lowering
rtPCR	Real-time PCR
SAM	Statin-associated muscle symptoms
SD	Standard deviation
SEARCH	Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine

siRNA	Small interfering RNA
SLCO1B1	Solute Carrier Organic Anion Transporter Family Member 1B1
SNPs	Single nucleotide polymorphisms
SRB1	Scavenger receptor B1
SREBP	Sterol regulatory element binding proteins
SREBP-1c	Sterol regulatory element-binding protein-1c
STATT	Simvastatin Treats Asians to Target
STELLAR	Statin Therapies for Elevated Lipid Levels Compared Across Doses to Rosuvastatin
T_a	Annealing temperature
T_m	Melting temperature
TAE	Tris-Acetate-EDTA
TCA	To come again-outpatient note
TC	Total cholesterol
TG	Triglyceride
TLC	Therapeutic lifestyle changes
TNP	Triple nucleotide polymorphisms
VLDL	Very low density lipoprotein
VOYAGER	Individual Patient Data Meta-Analysis of Statin Therapy in At-Risk Group: Effects of Rosuvastatin, Atorvastatin and Simvastatin
WOSCOPS	West of Scotland Coronary Prevention Study

LIST OF APPENDICES

HUBUNGAN ANTARA POLIMORFISME GENETIK DAN FAKTOR BERKAITAN DENGAN PROFIL LIPID DALAM KALANGAN PENGGUNA STATIN PESAKIT LUAR DI HOSPITAL USM

ABSTRAK

Statin, atau perencat reduktase 3-hidroksi-3-metilglutaril ko-enzim A (HMG CoA), sering digunakan untuk menurunkan aras lipid, terutamanya kolesterol lipoprotein berketumpatan rendah (LDL-c). Panduan rawatan mengesyorkan LDL-c sebagai sasaran utama rawatan pesakit aras lipid tinggi. Keberkesanan statin dalam menurunkan aras LDL-c dan kesan sampingannya adalah berbeza dalam sebilangan individu disebabkan oleh polimorfisme genetik dan faktor persekitaran. Walaubagaimanapun, kajian farmakogenetik antara faktor-faktor tersebut terhadap profil lipid pengguna statin di Malaysia masih terhad. Kami menilai impak polimorfisme genetik pesakit, latar demografi dan faktor klinikal terhadap kesan penurunan lipid oleh statin dalam kalangan pesakit aras lipid tinggi di Hospital Universiti Sains Malaysia (HUSM). Dalam kajian retrospektif rentas ini, sejumlah 229 pengguna statin dengan aras lipid tinggi telah direkrut dan genotip pesakit bagi lapan polimorfisme nukleotida tunggal (SNPs) dalam tujuh calon gen telah ditentukan menggunakan kaedah *polymerase chain reaction-restriction fragment length polymorphisms* (PCR-RFLP) dan disahkan oleh analisa jujukan. Menggunakan model genetik dominan, ujian t-test bebas telah digunakan untuk membandingkan jumlah kolesterol (TC), kolesterol lipoprotein berketumpatan tinggi (HDL-c), LDL-c dan trigliserida (TG), di mana keputusan yang menunjukkan perbezaan ketara telah distratifikasikan mengikut jantina. Suatu model regresi logistik berbilang binari telah

dibuat dengan LDL-c < 2.6 mmol/L pada titik akhir sebagai pembolehubah bersandar, manakala faktor penjelasan sebagai pembolehubah tidak bersandar. Kekerapan alel minor (MAF) ialah seperti berikut; *CETP* rs708272 = 0.39, *ABCG2* rs2231142 = 0.12, *ABCC2* rs717620 = 0.58, *APOE* E4 = 0.35, *GATM* rs9806699 = 0.63, *COQ2* rs4693075 = 0.96, dan *APOA5* rs662799 = 0.45. Hanya *CETP* rs708272 dan *COQ2* rs4693075 yang mempunyai MAF yang sama dengan populasi rujukan (i.e., populasi Asia Timur) yang diambil dari pangkalan data ENSEMBLE (P>0.05). Daripada keseluruhan SNPs, hanya *CETP* rs708272 and *ABCG2* rs2231142 yang berkait dengan parameter lipid sebelum rawatan. Sebelum rawatan statin, wanita pembawa *CETP* rs708272 dikaitkan dengan aras LDL-c yang lebih tinggi (4.02 ± 1.44 mmol/L vs 3.44 ± 0.84 mmol/L, P=0.007) dan TG yang lebih rendah (1.52 ± 0.63 mmol/L vs 1.90 ± 0.98 mmol/L, P=0.044). *ABCG2* rs2231142, dikaitkan dengan aras HDL-c yang lebih tinggi dalam kumpulan keseluruhan (1.38 ± 0.37 mmol/L vs 1.25 ± 0.26 mmol/L, P=0.035) dan wanita (1.49 ± 0.38 mmol/L vs 1.33 ± 0.27 mmol/L, P=0.047). Selepas pesakit mengambil rawatan statin, dua SNPs (*ABCC2* rs717620 and *APOA5* rs662799) telah dikaitkan dengan kesan anti-aterogenik. *ABCC2* rs717620 telah dikaitkan dengan penurunan aras TG yang lebih tinggi dalam kumpulan keseluruhan (1.48 ± 0.75 mmol/L vs 2.17 ± 1.14 mmol/L, P=0.009) dan lelaki (1.48 ± 0.85 mmol/L vs 2.40 ±0.91 mmol/L, P=0.006). Dalam kumpulan lelaki, pembawa *APOA5* rs662799 mempunyai aras HDL-c yang lebih tinggi (1.20 ± 0.25 mmol/L vs 1.07 ± 0.15 mmol/L, P=0.006) dan TG yang lebih rendah (1.42 ± 0.81 mmol/L vs 1.69 ± 0.75 mmol/L, P=0.038). Dalam regresi logistik berbilang binari, hanya pengguna pravastatin yang meramal kemampuan pesakit mencapai sasaran LDL-c<2.6mmol/L (P=0.040, OR=0.110). Rumusannya, *CETP* rs708272 dan *ABCG2* rs2231142 dapat menentukan perbezaan lipid sebelum rawatan statin dalam kumpulan wanita, manakala *ABCC2*

rs717620 dan *APOA5* rs662799 dapat menentukan perbezaan lipid selepas rawatan statin dalam kumpulan lelaki.

ASSOCIATION OF GENETIC POLYMORPHISMS AND THEIR ATTRIBUTING FACTORS WITH LIPID PROFILES AMONG OUTPATIENT STATIN USERS IN HOSPITAL USM

ABSTRACT

Statins, or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are routinely used to lower lipid levels primarily low-density lipoprotein cholesterol (LDL-c). Treatment guidelines recommend LDL-c as the primary target of therapy in hyperlipidaemic patients. Inter-individual variation in LDL-c-lowering efficacy and side effects of statins are due to genetic polymorphisms and environmental factors. However, pharmacogenetics studies on the effects of the aforementioned factors on the lipid profiles of statin users in Malaysia are still lacking. We evaluated the association of patient's genetic polymorphisms, demographic profiles, and clinical factors with lipid profiles among outpatient statin users from Hospital Universiti Sains Malaysia (HUSM). In a cross-sectional retrospective study, a total of 229 hyperlipidaemic statin users were recruited and the patients' genotypes for eight single nucleotide polymorphisms (SNPs) in seven candidate genes were determined using the polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method and validated by sequencing analysis. Using a dominant genetic model, an independent t-test was used to compare total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), LDL-c and triglycerides (TG), and the results with significant differences were stratified according to gender. A multiple binary logistic regression model was conducted, with LDL-c < 2.6 mmol/L at the endpoint serving as the dependent variable while other explanatory factors as

independent variables. Minor allele frequency (MAF) of the studied SNPs as follow; *CETP* rs708272 = 0.39, *ABCG2* rs2231142 = 0.12, *ABCC2* rs717620= 0.58, *APOE* E4 = 0.35, *GATM* rs9806699 = 0.63, *COQ2* rs4693075= 0.96, and *APOA5* rs662799= 0.45. Only *CETP* rs708272 and *COQ2* rs4693075 were matched to the MAF of the reference population (i.e., East Asian populations) obtained from the ENSEMBLE database (P>0.05). Of all SNPs genotyped, two SNPs (*CETP* rs708272 and *ABCG2* rs2231142) were associated with baseline lipid parameters. At the baseline before statin treatment, female minor allele carriers of *CETP* rs708272 were associated with higher LDL-c (4.02 ± 1.44 mmol/L vs 3.44 ± 0.84 mmol/L, P=0.007) and lower TG levels (1.52 ± 0.63 mmol/L vs 1.90 ± 0.98 mmol/L, P=0.044). *ABCG2* rs2231142 was associated with higher HDL-c levels in both overall (1.38 ± 0.37 mmol/L vs 1.25 ± 0.26 mmol/L, P=0.035) and females group (1.49 ± 0.38 mmol/L vs 1.33 ± 0.27 mmol/L, P=0.047). After the initiation of statin treatment, two SNPs (*ABCC2* rs717620 and *APOA5* rs662799) were associated with anti-atherogenic effects. In particular, *ABCC2* rs717620 was associated with significant reduction of TG levels in the overall (1.48 ± 0.75 mmol/L vs 2.17 ± 1.14 mmol/L, P=0.009) and males group (1.48 ± 0.85 mmol/L vs 2.40 ±0.91 mmol/L, P=0.006). Similarly in males, minor allele carriers of *APOA5* rs662799 resulted in higher HDL-c (1.20 ± 0.25 mmol/L vs 1.07 ± 0.15 mmol/L, P=0.006) and lower TG levels (1.42 ± 0.81 mmol/L vs 1.69 ± 0.75 mmol/L, P=0.038). In the multiple binary logistic regression analysis, only pravastatin users independently predicted patient's achieving LDL-target of <2.6 mmol/L (P=0.040, OR=0.110). In conclusion, *CETP* rs708272 and *ABCG2* rs2231142 determined the baseline lipid differences in females, whereas *ABCC2* rs717620 and *APOA5* rs662799 determined the lipid differences after statin initiation in males group.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Statin is also known as a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. It is one of the most commonly used lipid-lowering drugs in the market due to its ability to reduce LDL-c (Xu & Wu, 2020). There are many type of statins currently in the market, which are atorvastatin (Lipitor), simvastatin (Zocor), pitavastatin (Livalo), fluvastatin (Lescol), rosuvastatin (Crestor), pravastatin (Pravachol) and lovastatin (Mevacor). These statins are different in terms of their nature of productions, metabolisms in the liver, physical-chemical properties and specific activity of the statin itself (Ward et al., 2019).

The site of the action for statin takes place inside the liver, where it inhibits the cholesterol biosynthesis pathway called the mevalonate pathway (Stancu & Sima, 2001). It competitively inhibits HMG-CoA reductase enzymatic activity that converts HMG-CoA into mevalonate, a rate-limiting step in cholesterol biosynthesis (Buhaescu & Izzedine, 2007; Stancu & Sima, 2001). Therefore, the reduced production of mevalonate causes a decreased production of downstream products, mainly cholesterol (Buhaescu & Izzedine, 2007). To compensate for the reduced production of cholesterol, liver cells begin to increase expression of LDL-receptor (LDLR) on the cell surface, causing an increased uptake of plasma LDL-c (Ward et al., 2019). As a result, the level of plasma LDL-c is reduced (Adhyaru & Jacobson, 2018; Arrigoni et al., 2017; Ward et al., 2019).

Since plasma LDL-c level is one of the risk factors for cardiovascular diseases (CVD), a reduced level of plasma LDL-c is associated with the decreased incidence of CVD (Adhyaru & Jacobson, 2018). Although statin is commonly used, it is worth noting that statin efficacy in lowering LDL-c levels can vary between 5-70% among individuals, even when compliance is taken into account (Vladimirova-Kitova & Kitov, 2015). Various factors influence the statin efficacy such as genetic polymorphisms, demographic profiles and clinical factors.

Single nucleotide polymorphisms (SNPs) is the most common genetic polymorphism, estimated to occur around 1 in every 1000-2000 bases when two human chromosomes are compared (Sachidanandam et al., 2001). Researchers have found that SNPs may help to predict individual response to drugs, leading to a new branch of studies called pharmacogenomics (Alwi, 2005).

Based on previous studies, eight SNPs within seven genes had been selected for this study. The SNPs chosen are as follows: *CETP* rs708272 (involved in lipid metabolisms), *APOA5* rs662799; rs429358 and rs7412 in the APOE gene (for regulating either lipid synthesis or clearance from plasma), *ABCG2* rs2231142 and *ABCC2* rs717620 (for mediating the statin disposition) and *GATM* rs9806699 and *COQ2* rs4693075 (associated with statin toxicity). The rs708272 in cholesteryl ester transfer protein (CETP) gene has been associated with lipid metabolism, by regulating the reverse cholesterol transport (RCT). The RCT process is vital in regulating plasma lipid levels as excess cholesterol can be transported to the liver to be re-circulated or excreted (Trajkovska & Topuzovska, 2017). In term of regulating lipid synthesis and its clearance from plasma, *APOA5* rs662799 is involved in the synthesis of very-low-density lipoprotein (VLDL) in the liver. In addition, *APOA5* rs662799 also regulates the hydrolysis of lipoprotein and the remnant clearance from plasma (Forte & Ryan,

2015). Additionally, rs429358 and rs7412 both located in the multi-allelic apolipoprotein E (APOE) gene. The APOE gene is a component of several lipoproteins such as chylomicrons, chylomicron remnants, VLDL, and intermediate density lipoprotein (IDL). APOE gene plays a significant role in mediating their affinity binding with LDLR family on the hepatocytes, hence regulating the uptake and clearance of lipoproteins from plasma (Husain et al., 2021; Phillips, 2014). With regards to statin disposition, both rs2231142 and rs717620 are located in the ATP-binding cassette subfamily G member 2 (ABCG2) and ATP-binding cassette subfamily C member 2 (ABCC2) gene respectively. Both of these genes are crucial in facilitating statin excretion, affecting its pharmacokinetic property, therefore possibly influencing the statin bioavailability and its lipid-lowering effect (Liu et al., 2018; Prado et al., 2018; Wan et al., 2015). Both rs9806699 and rs4693075 are located in Glycine amidinotransferase (GATM) and Coenzyme Q2 (COQ2) gene respectively. These GATM and COQ2 genes were linked with statin-associated muscle symptoms (SAM), the most commonly reported statin side effects (Kitzmiller et al., 2016; Liu et al., 2020).

Besides genetic polymorphisms, patients' demographic profiles may also affect lipid parameters and statin efficacy. Based on a previous report, females tend to have a better HDL-c than males (Kim et al., 2011). Concerning statin efficacy, the previous study had shown that some SNPs exert their effects in a gender-specific manner (Liu et al., 2018; Lu et al., 2013). Furthermore, age factor may also play a role in influencing the statin efficacy, as evidence from the previous finding had reported the reduced efficacy of statin for the elderly group (Armitage et al., 2019).

Another factor that influences statin efficacy is clinical factors. Since there are various types of statins, each has a different structure that affects its solubility. The hydrophilic statin is more hepatoselective as it requires transporter protein on the hepatocytes, whereas the lipophilic statin can diffuse through passive diffusion (Climent et al., 2021; Maxwell et al., 2017). This difference in solubility may result in a better efficacy of statin treatment for a lipophilic statin, as evident from a previous study (Sakamoto et al., 2007).

Although many studies investigating these factors with statin efficacy have been conducted, only few studies have been focusing on our population (Punithavathi et al., 2009; Shuhaili et al., 2018). Since the statin efficacy may be different among populations, hence the current study remains relevant and vital. Therefore, the current study investigated the association between genetic polymorphisms, demographic profiles, clinical factors and statin efficacy in the Malaysian population. The current study would hopefully serve as an initial data and initiate further research of pharmacogenomics study of statin in Malaysian population.

1.2 Problems statement

Hyperlipidaemia (HPL) is one of risk factors of CVD (Adhyaru & Jacobson, 2018). Reducing lipid, mainly LDL-c level, has been recognised as an effective strategy in reducing CVD risk and mortality (Baigent et al., 2005; Law et al., 1994). According to the World Health Organization (WHO) (2019), CVD is the leading cause of death worldwide and has been estimated to cause 17.9 million death, accounting for 32.0% global death. Based on the previous Statistics on Causes of Death, Malaysia (2020), ischemic heart disease (IHD) had been reported as the main causes of death, representing 15.0% of death in Malaysia. Besides, report from National Health and

Morbidity Survey (NHMS), an increasing trend of HPL has been reported from 2006 to 2015 in Malaysia.

In the era of precision medicine, more attention is paid to the search for predictive markers of treatment efficacy and tolerability. Statin is one of the classes of drugs that could benefit from this approach because of their wide use and their incidence of adverse events. Statin has been recognised as first line of defence in primary and secondary prevention of HPL (Blais et al., 2021) suggesting that statin pharmacogenomics are vital in order to prescribe precision medicine in HPL management. However, its efficacy in reducing lipid levels can vary between individuals due to several factors including genetic polymorphisms, demographic profiles and clinical factors. Furthermore, limited pharmacogenomics studies on statin have been conducted in Malaysia so far. Therefore, studies on statin pharmacogenomics should be conducted especially in our Malaysian population to enhance understanding on statin treatment.

1.3 Hypothesis

Since the selected genetic polymorphisms had been shown to influence the lipid metabolisms, lipid synthesis and clearance, statin disposition and toxicity, theoretically, these genes can be used to determine statin efficacy in lowering lipid levels among HPL patients in our population. Therefore, it is hypothesized that the variability of lipid response among statin users is due to a variety of factors including, but not limited to, the abovementioned genetic polymorphisms, demographic profiles, and clinical factors.

1.4 Objectives

1.4.1 General objective

General objective of this study is to investigate the association between selected genetic polymorphisms, patients' demographic profiles, clinical factors and lipid-lowering effect of statins among HPL patients in HUSM, Kelantan.

1.4.2 Specific objectives

The specific objectives of the current study are as follows:

1. To assess the demographic profiles and clinical factors of outpatient statin users who attended Klinik Rawatan Keluarga (KRK), HUSM, and their lipid profiles.
2. To evaluate the association of the selected SNPs (*CETP* rs708272, *ABCG2* rs2231142, *ABCC2* rs717620, APOE gene, *GATM* rs9806699, *COQ2* rs4693075 and *APOA5* rs662799) underlying variability in lipid response in the HPL statin users.
3. To determine the gender-specific effects of the selected SNPs on lipid parameters among the subjects.
4. To determine the extent by which other attributing factors (e.g. type of statins, lipid profiles, concomitant drugs, patients' age and gender) contribute to the independent factor(s) associated with the achievement of LDL-c goal of <2.6 mmol/L in the hyperlipidaemic statin users.

CHAPTER 2

LITERATURE REVIEW

2.1 Hyperlipidaemia (HPL)

2.1.1 Definition of HPL

HPL is defined as elevated plasma lipid concentrations of TC, LDL-c or TG. The combination of these features, including low HDL-c level is called dyslipidaemia (Pirillo et al., 2021). There are two types of HPL, which are primary HPL (inherited due to genetic causes) and secondary HPL (due to other factors such as lifestyle or other diseases like hypothyroidism, nephritic syndrome) (Shattat, 2014). According to Fredrickson Classification, as listed in Table 2.1, primary HPL can further be classified into six categories concerning their elevated plasma lipoprotein (Beaumont et al., 1972; Shattat, 2014). Furthermore, the two most common forms of HPL are hypercholesterolaemia (HCL) and hypertriglyceridaemia (HTG) (Pirillo et al., 2021). HCL is associated with elevated LDL-c levels whereas HTG is associated with elevated TG levels and often accompanied by reduced HDL-c levels (Pirillo et al., 2021).

Table 2.1 Fredrickson Classification of Primary HPL

TYPE	DISORDER	PLASMA LIPOPROTEINS
I	Hyperchylomicronemia	Elevated chylomicrons
IIa	Familial/Polygenic Hypercholesterolaemia	Elevated LDL-c
IIb	Familial Combined Hyperlipidaemia	Elevated LDL-c Elevated VLDL
III	Familial dysbetalipoproteinaemia	Elevated IDL
IV	Familial hypertriglyceridaemia	Elevated VLDL
V	Endogenous hypertriglyceridaemia	Elevated VLDL Elevated chylomicrons

Table adapted from Shattat, 2014.

2.1.2 Lipid categories

Lipid Metabolites and Pathway Strategy (LIPID MAPS) Consortium defines lipid as hydrophobic or amphipathic small molecules, originated from entirely or partly, by condensation of thioesters or isoprene units (Fahy et al., 2011). Lipid classification had been made previously by The Lipid Library (http://lipidlibrary.aocs.org), Cyberlipids (http://www.cyberlipid.org) and the Lipid Bank (www.lipidbank.jp), which grouped lipid into three subclasses; simple group consisting of lipids that produce two separate units upon hydrolysis (e.g., acylglycerols); complex group, consisting of lipids that produce three or more separate units upon hydrolysis (e.g., glycerophospholipids); and derived lipids, consisting of alcohols and fatty acids derived by hydrolyzing the simple lipids (Fahy et al., 2011). A more recent update on lipid classification by the LIPID MAPS Consortium (https://www.lipidmaps.org/) had classified lipids by more comprehensive groups based on two fundamental building blocks (ketoacyl and isoprene), subsequently categorized lipids into eight different categories; fatty acyls, glycerolipids, glycerosphospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides (Liebisch et al., 2020). These lipid

categories by the LIPID MAPS Consortium, along with examples of each category's classes and subclasses, are given in Table 2.2.

Table 2.2 Lipid classification based on LIPID MAPS Consortium

LIPID CLASSIFICATIONS		
LIPID CATEGORIES	CLASSES	SUBCLASSES
Fatty acyls	Fatty acids and conjugates	Straight chain fatty acids
		Branched fatty acids
		Unsaturated fatty acids
Glycerolipids	Triradylglycerols	**TRIGLYCERIDE (TG)**
		Alkylglycerols
Glycerophospholipids	Glycerophosphocolines	Diacylglycerophosphocholines
Sphingolipids	Sphingoid bases	Sphinganines
		Sphingoid base analogs
Sterol lipids	Sterols	**CHOLESTEROL**
		Sterol esters
Prenol lipids	Isoprenoids	Polyterpenes
		Retinoids
Saccharolipids	Acylaminosugars	Acylaminosugar glycans
		Acyltrehaloses

Table adapted from https://www.lipidmaps.org/ .

Cholesterol (subclass of sterols) and TG (subclass of triradylglycerols) have been associated with CVD risk. This was demonstrated in a previous study involving seven countries (n=12763), which concluded a direct correlation between the average percentage of calorie intake from cholesterol and TG uptake and CVD following a follow-up period of five, ten and fifteen years (Welty, 2020).

2.1.2(a) Cholesterol

Cholesterol, a type of lipid, serves several vital roles in our body, including regulating the fluidity of cell membrane, precursor of bile acid, synthesis of steroid hormones and vitamin D (Alberts et al., 2002; Bikle, 2000; Hu et al., 2010; Norlin &

Wikvall, 2007). Cholesterol can be obtained through diet or synthesized *de novo*, predominantly in the liver and non-hepatic tissues on a smaller scale (Soliman, 2018). The cholesterol biosynthesis in the liver occurs through the mevalonate pathway, involving multiple enzymes, with acetyl-CoA as the precursor. The rate-limiting step of the mevalonate pathway is the conversion of HMG-CoA into mevalonate by the HMG-CoA reductase. The next crucial step is the conversion of farnesyl pyrophosphate (PP) into squalane, catalysed by the squalane synthase, which eventually produces cholesterol as the downstream product (Buhaescu & Izzedine, 2007). The mevalonate pathway is summarized in Figure 2.1

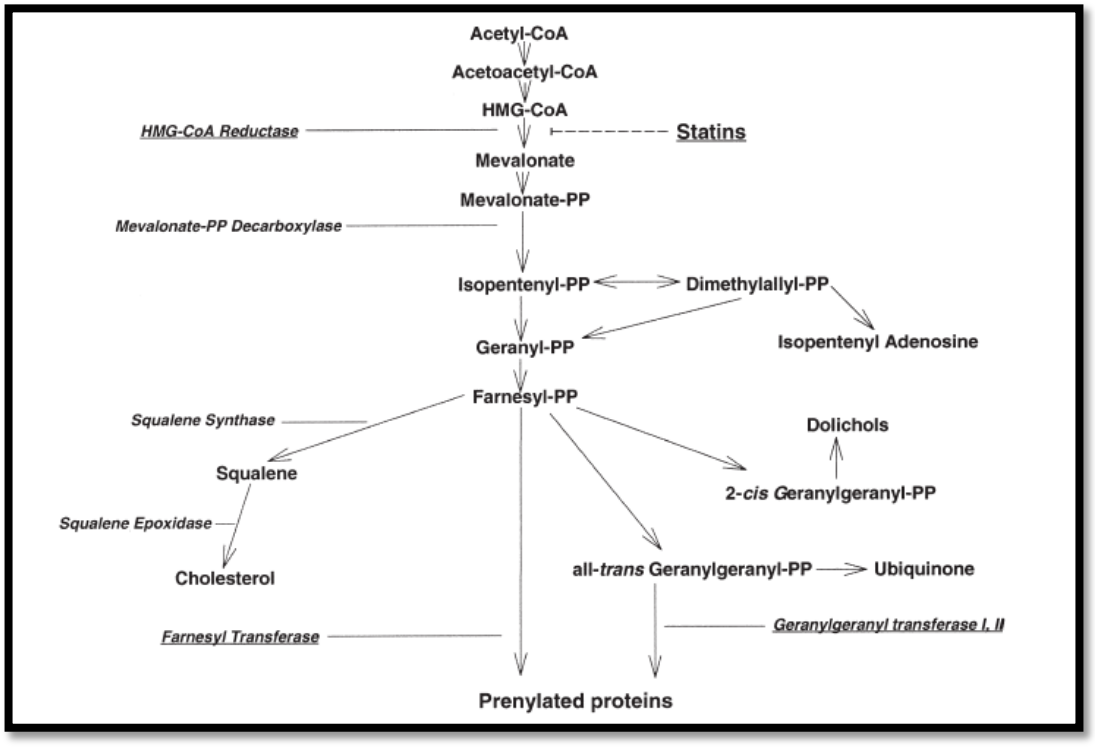

Figure 2.1 Mevalonate pathway in hepatocytes. This hepatic mevalonate pathway involves multiple enzymes and acetyl-CoA as a precursor. CoA, coenzyme A; PP, pyrophosphate. Adapted from Corsini et al., 1999.

Since cholesterol is non-soluble, the synthesized cholesterol is packaged inside the core of lipoprotein in the form of cholesteryl esters (the storage form of cholesterol). The structure of lipoprotein consists of the hydrophobic core (made up of cholesteryl esters and TG) surrounded by the outer hydrophilic membrane (consists of phospholipids, free cholesterol and apolipoprotein) (Figure 2.2). Cholesterol, in the form of cholesteryl esters together with TG, is transferred from the endoplasmic reticulum to the apo B-100, facilitated by microsomal triglyceride transfer protein (MTP), to form Very Low-Density Lipoprotein (VLDL) (Feingold & Grunfeld, 2000). VLDL is then secreted out of hepatocytes into the circulatory system to transport cholesterol to other cells and metabolised by lipoprotein lipase (LPL) (Brown & Goldstein, 1976; Feingold & Grunfeld, 2000). As VLDL is metabolised, fatty acids are liberated, and intermediate-density lipoprotein (IDL) is formed (Brown & Goldstein, 1976; Feingold & Grunfeld, 2000).

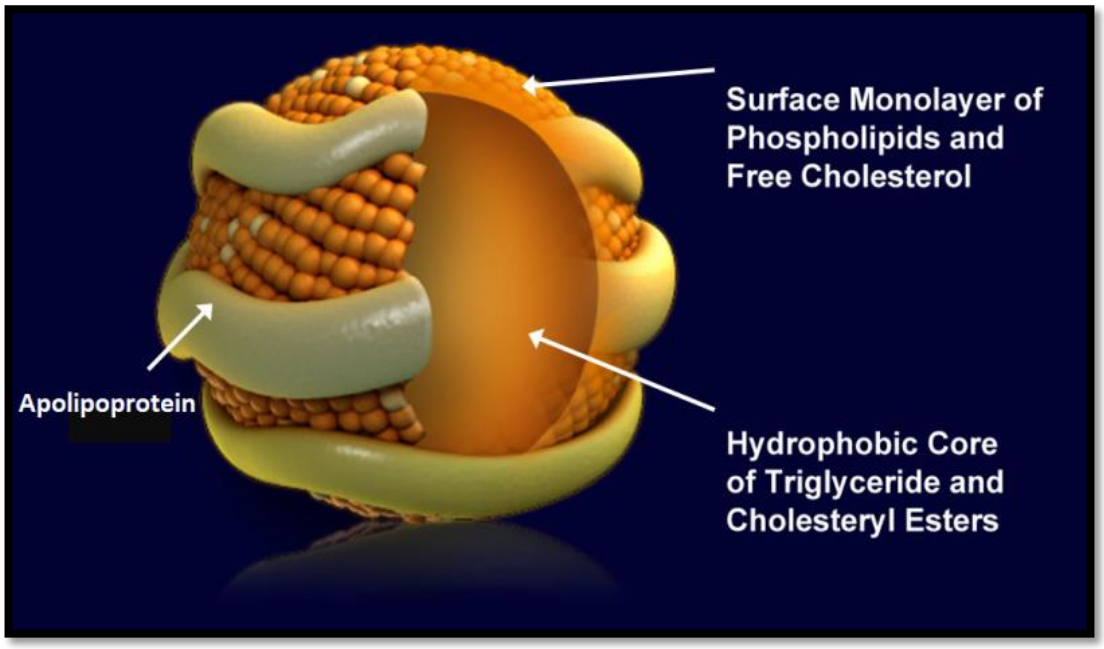

Figure 2.2 Lipoprotein structures. Adapted from Feingold & Grunfeld, 2000.

IDL contains less triglyceride, is enriched with cholesteryl esters, and can bind to LDLR on hepatocytes, therefore facilitating its clearance from circulation (Brown & Goldstein, 1976; Feingold & Grunfeld, 2000). In addition, some IDL are converted into LDL by hepatic lipase, which hydrolyses the remaining TG inside IDL and further reduces its TG content (Feingold & Grunfeld, 2000). Therefore, LDL is enriched with cholesteryl esters and removed from circulation by either hepatic LDLR or taken up by extrahepatic tissues (Brown & Goldstein, 1976; Feingold & Grunfeld, 2000). This whole process of distributing the synthesized cholesterol in hepatocytes to other tissues is called the endogenous cholesterol metabolism pathway.

Besides, excess cholesterol from extrahepatic tissues is transported back to hepatocytes in RCT. This pathway begins with the production of apolipoprotein A-I (APOA1) by the liver and intestine, which interacts with the ATP binding cassette subfamily A member 1 (ABCA1) transporter, to generate pre-ß HDL (Ahsan et al., 2014; Luo et al., 2020). Then, excess cholesterol in peripheral cells is exported out into pre-ß HDL, converted into mature HDL-c via the esterification process (Ahsan et al., 2014). Cholesterol in HDL-c is then taken into the liver, either by direct uptake via hepatic class B scavenger receptor B1 (SRB1) or indirect uptake by hepatic LDLR after being transferred to LDL by CETP (Ahsan et al., 2014; Feingold & Grunfeld, 2000). Hence, RCT is necessary to restore excess cholesterol in peripheral cells to the liver for re-circulation or removal.

2.1.2(b) Triglycerides (TG)

TG also serves multiple functions in our body, such as being the source of energy, heat thermal insulation, carrying lipid-soluble vitamins and structural component of membrane (Aguilera-Méndez et al., 2013). TG can either be synthesized

within the hepatocytes, circulated by VLDL in the endogenous pathway, as mentioned in the previous section 2.1.2 (a) or consumed through diet (Kindel et al., 2010; Shepherd, 2001). From our diet, TG is absorbed by intestinal cells and packed together with cholesteryl esters (obtained from bile) before entering the circulation as a component of chylomicrons (Feingold & Grunfeld, 2000; Shepherd, 2001). Chylomicrons are then metabolised by LPL in muscle and adipose tissue, liberating free fatty acids (Feingold & Grunfeld, 2000). The chylomicron remnants formed subsequently are lesser in size, enriched with cholesteryl esters and have also acquired APOE from HDL-c (Feingold & Grunfeld, 2000). APOE protein on chylomicron remnants binds to LDLR or low-density lipoprotein related receptor (LRP) on hepatocytes, therefore mediating TG clearance (and cholesteryl esters) from plasma (Feingold & Grunfeld, 2000; Shepherd, 2001).

This whole process of delivering dietary fats and cholesterol from bile is called the exogenous lipid metabolism pathway. The summary of the endogenous and exogenous pathways of lipid metabolisms are shown in Figure 2.3.

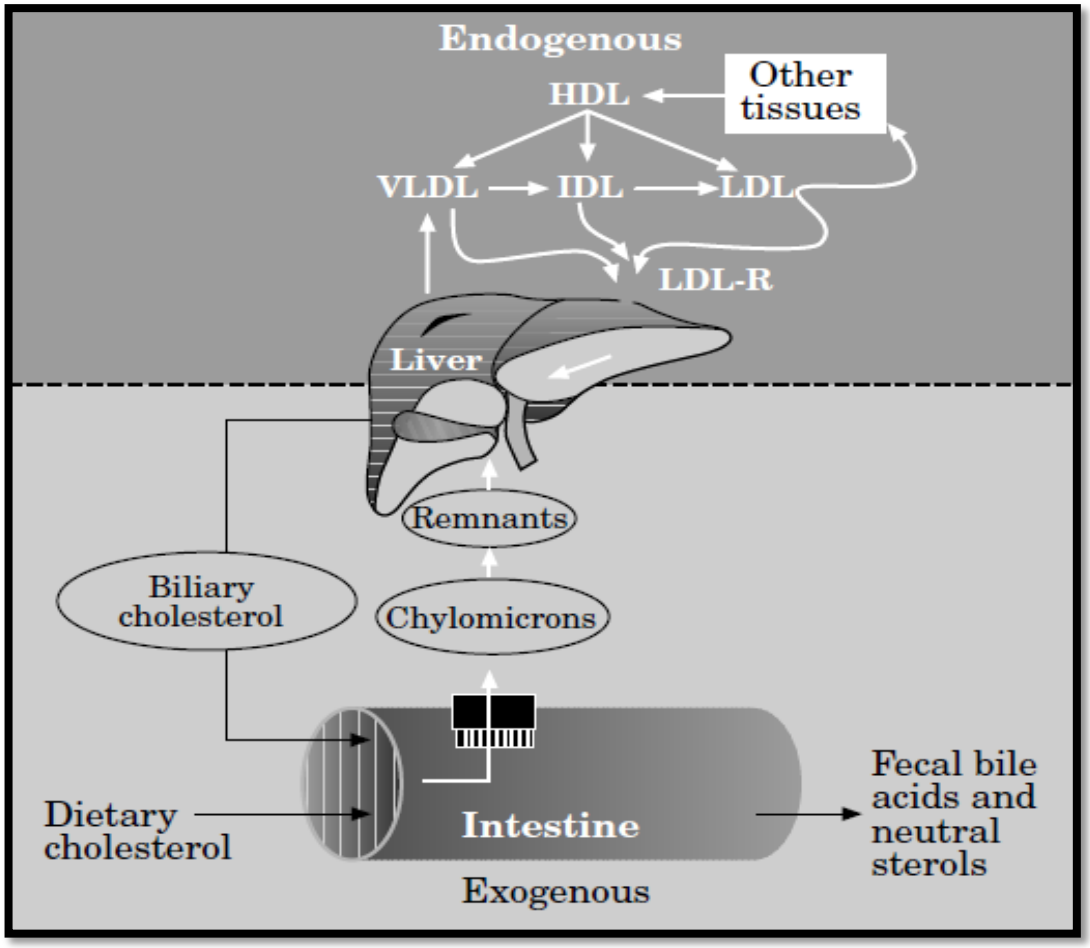

Figure 2.3 Exogenous and endogenous pathway of cholesterol metabolisms in human body. Adapted from Shepherd, 2001.

2.1.3 Prevalence of HPL

According to World Health Organization (https://www.who.int/), in 2008, the global prevalence of elevated TC aged 25 and above was approximately 39.0%. However, HPL prevalence varied in different countries in Southeast Asian region, such as the Philippines with 46.9% in 2013, Indonesia with 35.8% in 2008, and Singapore with 17.4% in 2010, although these discrepancies could be due to different cut-off values of abnormal lipid levels (Lin et al., 2018). Focusing on Malaysian region, the increasing prevalence of HPL had been reported by the National Health and Morbidity Survey (NHMS), from 20.7% in 2006 to 35.1% in 2011 and 47.7% in 2015 (Mat Rifin

et al., 2018). Despite the decrease in HPL prevalence in overall population (38.1%) as shown by the latest NHMS report in 2019, it was worth noting that the highest prevalence of HPL had been recorded in Kelantan population (51.1%).

2.1.4 Consequences of HPL

HPL had been associated with CVD risk, as previously reported in a study using the Framingham Offspring Cohort, without incident of coronary heart diseases (CHD) (n=1478). The study concluded that CHD rates were significantly elevated ($P<0.001$) with prolonged exposure of HPL; 4.4% in subjects with no exposure of HPL, 8.1% in subjects with 1-10 years of HPL exposure and 16.5% in subjects with 11-20 years of HPL exposure (Navar-Boggan et al., 2015). Moreover, HPL had also been associated with CVD mortality, as demonstrated by a meta-analysis study involving 32 cohort studies from the Asia-Pacific Cohort Studies Collaboration (n=372741), in which all TC, LDL-c, HDL-c and TG levels were independently associated with CVD death (all $P<0.05$) (Barzi, 2005). Another meta-analysis involving 61 prospective studies (n=892337) had also shown the association between TC levels and ischemic heart diseases (IHD), with 1.0mmol/L reduction of TC, was related to reduced risk of IHD mortality for both sexes in the age of 40-49 (Hazard ratio=0.44), 50-69 (Hazard ratio=0.66) and 70-89 (Hazard ratio=0.83) (MacMahon et al., 2007).

2.1.5 Treatment of HPL

2.1.5(a) Therapeutic lifestyle changes (TLC)

Since HPL is one of the risk factors of CVD, treatment of HPL seems necessary to reduce the risk and deaths caused by CVD. An analysis done on 10 prospective

cohort studies showed that reducing cholesterol levels by 0.6mmol/L (about 10%) caused a decrease in the incidence of IHD depending on the age (Law et al., 1994). This evidence was also supported by a meta-analysis of 14 randomized trials in Cholesterol Treatment Trialists' (CTT) Collaborators (n=90056), which demonstrated that 1.0mmol/L of LDL-c reduction had reduced the coronary mortality by 19.0% (Risk ratio=0.81, P<0.0001) (Baigent et al., 2005).

The guidelines in the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) recommended the LDL-c as the primary target of lipid reduction, and highlights two major modalities in reducing LDL-c, through therapeutic lifestyle changes (TLC) or by lipid-lowering drugs (Safeer & Ugalat, 2002). Table 2.3 summarises LDL-c goal and cut off points for TLC and lipid-lowering drugs for each risk category based on NCEP ATP III guidelines. According to the guidelines, medical doctors or health practitioners often advise on initiating TLC to assist in achieving LDL-c goal for 12 weeks before considering drug therapy (Safeer & Ugalat, 2002). TLC includes controlling dietary intake, physical activity and weight loss. Concerning dietary intake, the NCEP ATP III guideline recommends the intake of monounsaturated (up to 20% of total calories), polyunsaturated fats (up to 10% of total calories), a controlled cholesterol intake of <200mg/dL (5.2mmol/L) and the addition of plant stanols (2g/day). Besides, aerobic exercise is also recommended as a way to lower TG levels because it leads to weight loss, which could also help to lower LDL-c levels (Safeer & Ugalat, 2002).

Table 2.3 LDL-c goals and cut off points for TLC and lipid-lowering drugs

Risk categories	LDL-c target (mg/dL)	LDL-c level to initiate TLC (mg/dL)	LDL-c level to consider drug therapy (mg/dL)
CHD or CHD risk equivalent (10-year risk>20%)	<100 (2.60mmol/L)	≥100	≥130
Two or more risk factors (10-year risk ≤20%)	<130 (3.35mmol/L)	≥130	≥130 (10-year risk 10-20%)
			≥160 (10-year risk <10%)
0-1 risk factor	<160 (4.15mmol/L)	≥160	≥190

Table adapted from NCEP ATP III guidelines.

2.1.5(b) Lipid-lowering drugs

If TLC treatment of HPL is less effective and LDL-c levels are still elevated above the optimum level, drug therapy could be initiated. Statin, is the first line of defence for primary and secondary prevention of CVD (Blais et al., 2021). The use of statin as the first line of defence had been demonstrated by the trending usage of statin in 2018, in which statin remains as the most widely used lipid-lowering drug by approximately 145.8 million people (~2.6% from the study population that covered 74.0% of the world population in 2018) (Blais et al., 2021). This was evident by a meta-analysis involving 84 randomized controlled trials (n=246706), which concluded that statin had been associated with reduced CVD mortality as compared to placebo (OR=0.83), whereas no significant association was seen for other lipid-lowering drugs such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and ezetimibe (Zhao et al., 2019).

Because statins were thought to be safe and well-tolerated, they have been widely used as lipid-lowering medications. However, discontinuation and non-adherence of statin treatment remain a problem in the treatment of HPL, mainly because of the SAM (Ward et al., 2019). High dosage of simvastatin (i.e., 80 mg/d) has been related to an unacceptably increased risk of SAM compared to 20 mg/d simvastatin (Risk ratio=26.6, $P<0.0001$), as demonstrated by the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group (n=12064), (Webster et al., 2010). Other side effects of statin include new onset of type 2 diabetes mellitus (DM), which is common in high-risk patients, as previously shown in the Justification for the Use of Statin in Prevention (JUPITER) trial (n=17603), which concluded a 28.0% increase in diabetes for high-risk patients ($P=0.01$) (Ridker et al., 2012).

Besides DM, analysis of cases from the Spanish Hepatoxicity Registry (n=858) demonstrated the association between statin and hepatotoxicity, with statins accounting for 5.5% (n=47) of the drug-induced liver injury (DILI) cases (Björnsson, 2017). However, positive effect of statin in reducing the global burden of CVD still outweighs its adverse side effects as the prevalence of statin side effects varies among statin users, depending on statin used, dosages and interacting drugs (Armitage, 2007).

Other treatments such as ezetimibe, in addition to statins, can be used alone or in combination with statin to treat HPL. Ezetimibe reduces dietary cholesterol absorption by binding to the Niemann-Pick C1-Like 1 (NPC1L1) receptor in intestinal cells (Soran et al., 2018) and has been recommended to be used as an adjunct to statin treatment in statin intolerance group by the European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS). However, its effectiveness in

reducing LDL-c levels is generally 10-15% lower than that of statins (Soran et al., 2018).

Bile acid sequestrating agents work by resisting the reabsorption of bile acid in the ileum, which increases hepatic cholesterol requirement, causing the hepatocytes to increase the expression of LDLR to increase the uptake of LDL-c, thereby reducing plasma LDL-c level (Soran et al., 2018). Its efficiency in reducing LDL-c is generally around 15-30%, according to NCEP ATP III (Cleeman, 2001). The examples of bile acid sequestrating agents available in the market are cholestyramine, colestipol and colesevelam.

Fibrates are amphipathic carboxylic acid, mainly to reduce TG level, which works by reducing the production of TG in the liver by stimulating the uptake of cellular fatty acids, followed by conversion into acyl-CoA in the liver, resulting in reduced availability of fatty acids for TG synthesis (Staels et al., 1998). Besides, fibrates can also increase the production of HDL-c and RCT by increasing the production of APOAI and apolipoprotein-AII (APOAII) in the liver, which is the major component of HDL (Feingold & Grunfeld, 2000; Staels et al., 1998). The efficiency of fibrates against the HTG and hypoalphalipoproteinaemia (HDL deficiency) can be seen in the subgroup analysis of Helsinki Heart Study, which found that fibrates had the best preventive efficacy in about 10% of the study population with LDL: HDL ratio of >5 and TG level of 2.3 mmol/L (Staels et al., 1998). In the event that statins are ineffective, fibrates are frequently used as an alternative. Examples of fibrates available in the market are gemfibrozil, fenofibrate and clofibrate.

2.2 Statin treatment

2.2.1 Discovery of statin

Akira Endo first discovered statin in the 1970s, called compactin, extracted as a compound from fungi *Penicillium Citrinum* that can inhibit HMG-CoA reductase activity (Endo, 2008). Merck's further studies produced lovastatin, which was extracted from another type of fungi *Aspergillus Tereus* (Endo, 2008). The discovery of statin was halted as clinical development of compactin was suspended in August 1980 (Endo, 2008). The suspension was later lifted as a successful test of familial hypercholesterolaemia (FH) was reported, allowing Merck to continue its clinical development of lovastatin, which was later approved by the Food and Drug Administration (FDA) (Endo, 2008). This was later followed up by simvastatin and pravastatin by the Sankyo company (Endo, 2008). More statin derived synthetically was developed, including fluvastatin, cerivastatin, atorvastatin, rosuvastatin, and pitavastatin, although cerivastatin was withdrawn from the market due to its reported side effect of myopathy (Endo, 2008). Statin is currently used as the first line of drug therapy against HPL all over the world (Blais et al., 2021).

2.2.2 Types of statin

Statin can be classified according to a few characteristics; how the statin is obtained (either produced by fungal fermentation, semi-synthetic or fully synthetic), how they are metabolised in the liver, their physical-chemical properties and their specific activity (Stancu & Sima, 2001). Lovastatin and pravastatin are produced by fungal fermentation, simvastatin is semi-synthetic which are derived by chemical modification of lovastatin side chain, whereas atorvastatin, fluvastatin, rosuvastatin

and pitavastatin are derived synthetically (Manzoni & Rollini, 2002; Stancu & Sima, 2001).

Figure 2.4 below shows the general structure of statin which is made up of two key components, naphthalene ring and ß-hydroxylactone. Naturally produced statins have pretty similar chemical structures, mainly differing in side chains attached to C6 and C8 of the naphthalene ring system (Manzoni & Rollini, 2002). Lovastatin has a 6-α methyl group at C6 and methylbutyric side chain at C8. Pravastatin has a hydroxyl group attached at the C6 position instead of methyl and has ß-hydroxylactone in 6-hydroxy sodium salt form, whereas simvastatin has an additional methyl group at 2' position of the side chain at C8 (Manzoni & Rollini, 2002; Matusewicz et al., 2015). When compared to naturally produced statin, synthetically produced statin has different structures. The only thing that the two groups has in common is the HMG-CoA-like moiety, which inhibits HMG-CoA reductase (Manzoni & Rollini, 2002; Matusewicz et al., 2015). Besides, synthetic statins are obtained in hydroxyl acid form, and they share a common fluorophenyl group (Matusewicz et al., 2015). Fluvastatin is derived from mevalolactone; atorvastatin is pyridine derivatives; pitavastatin has a unique cloropropyl group, whereas rosuvastatin has a methanesulfonamide group (Matusewicz et al., 2015). Figure 2.5 below shows the chemical structure of each statin.

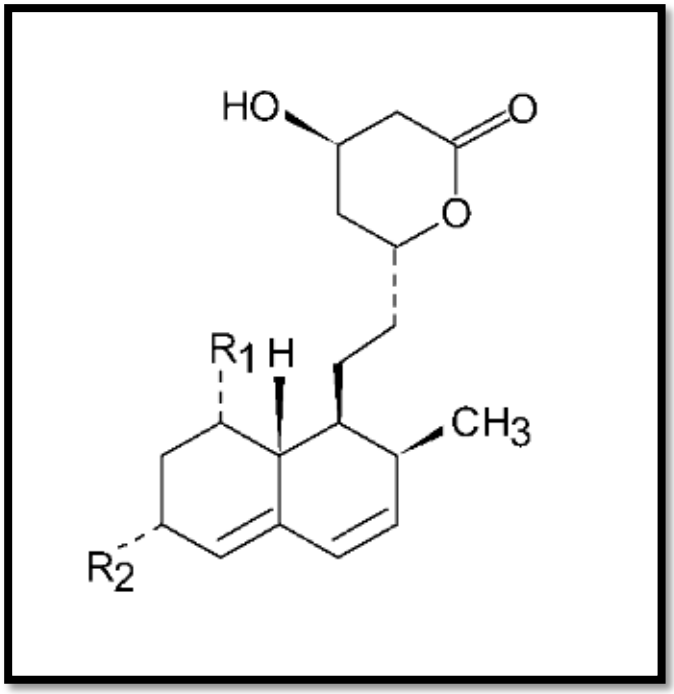

Figure 2.4 Basic structure of statin. Statin is made up of naphthalene ring system and ß-hydroxylactone. R1 represents the side chain at C8, whereas R2 represents the side chain at C6. Adapted from Matusewicz et al., 2015.

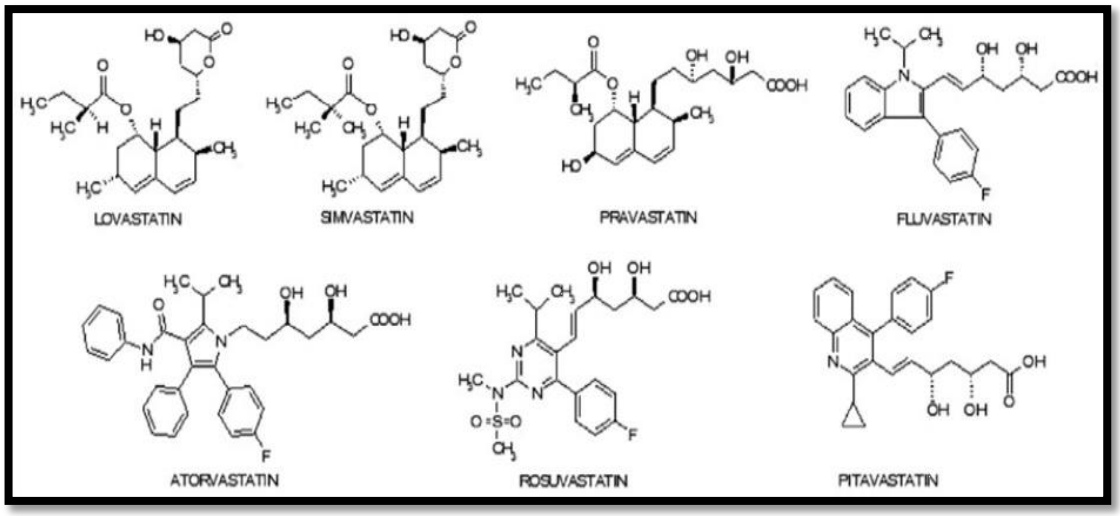

Figure 2.5 Structures of each organic, semi-synthetic and synthetic statin. Adapted from Fedacko et al., 2017.

Besides, statins can also be grouped based on their physical-chemical properties. For example, lovastatin, simvastatin, fluvastatin, atorvastatin, pitavastatin are lipophilic statins, whereas pravastatin and rosuvastatin are hydrophilic statins due to their polar hydroxyl group and methanesulfonamide group in its respective side chain (Schachter, 2005; Stancu & Sima, 2001).

Most statins are metabolized by the cytochrome P450 (CYP450), family of enzymes in the liver (Schachter, 2005). Lovastatin, simvastatin, atorvastatin are metabolized by cytochrome P450 3A4 (CYP3A4) isoenzymes, whereas fluvastatin is metabolized by cytochrome P450 2C9 (CYP2C9) isoenzyme (Schachter, 2005). Rosuvastatin and pitavastatin undergo minimal oxidative metabolism by CYP450 pathway (Schachter, 2005). Pravastatin is mainly metabolized in the stomach rather than CYP450 pathway in the liver (Hatanaka, 2000).

2.2.3 Mechanisms of action

Statin works inside the liver, where it competitively inhibits HMG-CoA reductase activity from converting HMG-CoA into mevalonate, a rate-limiting step in cholesterol biosynthesis (Buhaescu & Izzedine, 2007; Stancu & Sima, 2001). The HMG-CoA-like moiety structure allows statin to bind to HMG-CoA binding site, hindering other substrates binding to HMG-CoA binding sites (Istvan, 2003). The binding of statin to the active site also alters the conformation of HMG-CoA reductase, therefore preventing the enzyme from attaining its functional structure, making statin more specific and efficient (Stancu & Sima, 2001). As the conversion of HMG-CoA is reduced, the production of mevalonate also decreases.

Consequently, cholesterol, which is a downstream product of the mevalonate pathway, is also reduced. To compensate for decreased cholesterol levels, hepatocytes induce protease activation to cleave sterol regulatory element binding proteins (SREBP), which is then transported into the nucleus, where it activates transcription of LDLR (Luo et al., 2020; Stancu & Sima, 2001). As a result, the expression of LDLR on hepatocytes is elevated, increasing the uptake of circulating LDL-c and its precursors (VLDL and IDL), leading to a reduced plasma LDL-c level (Stancu & Sima, 2001).

2.2.4 Statin efficacy in determining lipid profiles/responses

According to NCEP ATP III, LDL-c should be the primary treatment target in lipid-lowering therapy to reduce CVD. Based on evidence from the clinical trial, statin remains the first-line drug to treat HPL (Feingold, 1999; Schachter, 2005). According to CTT Collaboration Study, 90 056 participants recruited in 14 randomized statin trials demonstrated a 12% reduction in all-caused death rates, per mmol/L reduction of LDL-c (Baigent et al., 2005). The mean LDL-c differences ranged from 0.35 mmol/L to 1.77 mmol/L at one year within all the trials (Baigent et al., 2005).

However, it is worth noting that statin efficacy in reducing LDL-c can vary from 5 to 70% between individuals, even when compliance is taken into account (Vladimirova-Kitova & Kitov, 2015). Several factors can influence statin efficacy in reducing LDL-c levels which are genetic polymorphisms, demographic profiles, and clinical factors.