ORIGINAL RESEARCH

Association of Drug-Metabolizing Enzyme and Transporter Gene Polymorphisms and Lipid-Lowering Response to Statins in Thai Patients with Dyslipidemia

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Purpose: Statins are increasingly widely used in the primary and secondary prevention of cardiovascular disease. However, there is an inter-individual variation in statin response among patients. The study aims to determine the association between genetic variations in drug-metabolizing enzyme and transporter (DMET) genes and lipid-lowering response to a statin in Thai patients with hyperlipidemia.

Patients and Methods: Seventy-nine patients who received statin at steady-state concentrations were recruited. Serum lipid profile was measured at baseline and repeated after 4-month on a statin regimen. The genotype profile of 1936 DMET markers was obtained using Affymetrix DMET Plus genotyping microarrays.

Results: In this DMET microarray platform, five variants; *SLCO1B3* (rs4149117, rs7311358, and rs2053098), *QPRT* (rs13331798), and *SLC10A2* (rs188096) showed a suggestive association with LDL-cholesterol-lowering response. HDL-cholesterol-lowering responses were found to be related to *CYP7A1* gene variant (rs12542233). Seven variants, *SLCO1B3* (rs4149117, rs7311358, and rs2053098); *SULT1E1* (rs3736599 and rs3822172); and *ABCB11* (rs4148768 and rs3770603), were associated with the total cholesterol-lowering response. One variant of the *ABCB4* gene (rs2109505) was significantly associated with triglyceride-lowering response.

Conclusion: This pharmacogenomic study identifies new genetic variants of DMET genes that are associated with the lipid-lowering response to statins. Genetic polymorphisms in DMET genes may impact the pharmacokinetics and lipid-lowering response to statin. The validation studies confirmations are needed in future pharmacogenomic studies.

Keywords: drug-metabolizing enzymes, drug transporters, gene polymorphisms, statin

Introduction

Dyslipidemia is the main risk factor for first and recurrent atherosclerotic cardiovascular disease (ASCVD) events. Atherosclerotic cardiovascular disease includes coronary artery disease, cerebrovascular disease, and peripheral artery

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disease, all of which can lead to death if left untreated. It is one of the major causes of death globally and the top cause of death in Thailand.[1] Statins (HMG-CoA reductase inhibitors) are drugs that are effective for reducing lipid levels and ASCVD prevention.[2,3] However, there is an inter-individual variation in statins' response among patients. It has been reported that 48.2% of the patients did not reach the therapeutic low-density lipoprotein (LDL) cholesterol target, and only 35.5% of the patients achieved the therapeutic goal.[4,5] The variation of individual responses to statin treatment may not only be owing to environmental factors such as medication adherence, exercise, and diet but also because of genetic factors. Polymorphisms of genes that involve in the regulation of lipid metabolisms such as *apolipoprotein E (apo E), cholesteryl ester transfer protein (CETP)*, and *liver X receptors (LXR)* have been reported to correlate with the efficacy of statin and susceptibility to cardiovascular disease.[6–11] Several studies have revealed that the genetic variants of the pharmacokinetics-related genes result in the diversity of statin efficacy among individuals.[12–14] Since statins have the primary site of action and metabolism in the liver, where they inhibit HMG-CoA reductase, the genetic variations of Phase I and II drug-metabolizing enzymes and drug transporter genes may have a critical role in the concentration of statins within hepatocytes and plasma, and consequently clinical efficacy to the treatment of statin. Genetic variations on influx transporters and efflux transporters such as *solute carrier organic anion transporter family, member 1B1 (SLCO1B1), ATP binding cassette subfamily B member 1* (*ABCB1*), and *ATP binding cassette subfamily G member 2 (ABCG2)* genes polymorphisms have been reported to be associated with the alteration of the pharmacokinetics of statin transport and the reduction of the lipid-lowering effect of statins.[15–19] In addition, genetic variants of the drug-metabolizing enzyme such as *cytochrome P450 3A4* (*CYP3A4*) and *cytochrome P450 3A5 (CYP3A5)* gene polymorphisms have been suggested that related to the lipid-lowering efficacy of statins.[20,21] However, the influences of genetic polymorphisms have been inconsistently replicated, and also genetic variations are related to ethnicity; the interethnic differences can be a noteworthy factor in drug response.[22,23] The study of Na Nakorn et al reported that the allele frequencies of the c.388A>G variants in the *SLCO1B1* gene were different among the Thai population and Asian Indians and Caucasians.[24] The Affymetrix Drug Metabolizing Enzyme and Transporters (DMET) microarray platform has been demonstrated that it is valuable in studying interethnic in drug response.[25] The Affymetrix DMET platform is a useful tool for simultaneous genotyping of a large number of genetic polymorphisms, which investigates a total of 1936 genetic markers (1931 SNPs and 5 CNVs), over 231 pharmacokinetics-related genes. A broad approach in evaluating the influence of multiple DMET gene variations on the lipid-lowering efficacy of statins may offer a better understanding of their etiological mechanisms. However, there have not been any association studies between genetic variants of DMET genes and the lipid-lowering response of statin in the Thai population. The objective of this study was to investigate the association between genetic polymorphisms of the DMET genes and lipid-lowering response of statin in the Thai population by using the Affymetrix DMET Plus genotyping microarrays.

Materials and Methods
Eligible Patients
Seventy-nine patients subjected to treatment with statins were recruited from the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. For this retrospective cohort study, patients (20–80 years old) received statins for at least 3 months. Serum lipid profile was measured at baseline (the first time of sampling) and repeated after 4-month on a statin regimen. The exclusion criteria were (1) patients with liver disease, renal disease, familial hypercholesterolemia, uncontrolled thyroid disease, and/or malignant diseases; (2) patients receiving concomitant medications that could potentially affect the lipid profile and pharmacokinetics of statins; (3) adjusted dose during treatment; and (4) non-compliant patients. The study was approved by the Ramathibodi Ethics Committee (MURA2019/735). All methods were carried out per the Declaration of Helsinki.

DMET Plus Array and Quality Control
Genomic DNA was extracted from EDTA whole blood using the MagNA Pure automated extraction system (Roche Applied Science, Penzberg, Germany). The quality and quantity of genomic DNA were measured by using NanoDrop Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

Genotype analysis using the DMET Plus platform is based on an efficient and comprehensive molecular inversion probe (MIP) technology (Affymetrix Inc., Santa Clara, CA, USA). The Affymetrix DMET[TM] Plus microarray enables individuals to test a total of 1936 genetic markers including 1931 SNPs and 5 copy number variations (CNVs) across 231 genes having functional significance in drug pharmacokinetics.[26] The PCR products from MIP technology were fragmented by fragmentation enzyme and then hybridized on DMET microarray.[27,28] Affymetrix fluidic stations were used to wash and stain the DMET arrays. DNA samples on the DMET Plus array were scanned with the Affymetrix GeneChip R Scanner3000 (AffymetrixInc, Santa Clara, CA, USA). The genotyping profiles of DMET SNPs were generated by DMET™ Console Software[®] (Version 1.3; Affymetrix Inc.). Quality control of genotyping data was checked by using the R package "GenABEL" and Haploview software.[29] Individuals with a <95% genotyping call rate were excluded. SNPs with minor allele frequency (MAF) <0.05, SNPs with more than 5% missing genotypes, a deviation from Hardy–Weinberg equilibrium (HWE) at $P > 0.05$, and variants on chromosome X were also excluded.

Statistical Analysis

The demographic and phenotypic measurements were analyzed using the SPSS (version 22.0). The normal distribution of the data was tested by Kolmogorov–Smirnov test. As the data are not normally distributed, continuous data were reported as the median and interquartile range (Q1–Q3). Association between types of statins and percent changes in lipid profile was analyzed using Kruskal–Wallis Test. P-value <0.05 was considered significant. Multidimensional scaling (MDS) plot and association analysis between DMET markers and percent changes in lipid profile were estimated using Cochran–Armitage test for trends in the GenABEL package of R version 3.1.1.[30] The P-value adjusted after Bonferroni correction was set at 2.5×10^{-5}. However, as an exploratory study, genetic markers were selected as prominent ones in the association analysis with a P-value less than 0.01, corrected for possible inflation due to some degree of stratification. Pairwise linkage disequilibrium analysis was performed using Haploview 4.2.

Results

Demographic Data and Clinical Characteristics

Seventy-nine participants, the sample group, were mostly female (n = 48; 60.80%). The median age of patients was 63.00 years (IQR: 55.00–72.00). Simvastatin was the most prescribed (81.01%) followed by atorvastatin (12.66%), and pitavastatin (6.33%), respectively. For statins dosing, the percentage of the patients treated with statins 2 mg/day, 10 mg/day, 20 mg/day, and 40 mg/day were 6.33%, 50.63%, 27.85%, and 15.19%, respectively. Comorbidity among patients was hypertension (29.11%) and diabetes mellitus (25.32%) (Table 1). There was no association between types and doses of statins and percentage change in lipid profile as shown in Table 2.

Association Between DMET SNPs and Lipid-Lowering Responses to Statin

After filtering 80 samples and 1931 SNP markers from DMET[TM] Plus GeneChip microarray platform (Affymetrix Inc., Santa Clara, CA, USA) with the quality control parameters, one sample was removed due to low call rate (<95%), 194 (10.04%) markers were excluded because of an overall call rate of less than 95%. For the remaining 1737 markers, 1232 (70.92%) markers were removed owing to minor allele frequency (MAF) less than 5%. Also, 46 markers on the X chromosome and 31 markers with P-value <0.05 in HWE were excluded as well. As a result, only 79 samples (98.75%) and 419 (21.69%) markers of 1931 markers were included for final analysis. A multidimensional plot did not show any clear clusters, which means that no population stratification existsand also there were no differences in allele frequencies among the studied population (see Supplementary Figure S1).

The DMET SNPs showed significant associations with the percentage change in lipid profile with the P-value <0.01. The top significant SNPs associated with lipid profile were shown in Table 3 and Figure 1. Of all the DMET SNPs, five SNPs of three genes (*solute carrier organic anion transporter family member 1B3* (*SLCO1B3*); c.334G > T (rs4149117), c.699A > G (rs7311358), and c.1557G > A (rs2053098), *quinolinate phosphoribosyltransferase* (*QPRT*); T>G (rs13331798), and *solute carrier family 10 member 2* (*SLC10A2*); c.511G>T (rs188096) were found to be associated with the percentage change in LDL-cholesterol after 4 months of statin treatment (P-value <0.01) (Table 3 and

Table 1 Demographic Data and Clinical Characteristics of Study Subjects (n=79)

Parameters	Median (IQR)
Gender (Male/Female), n (%)	31 (39.20)/48 (60.80)
Age, years	63.00 (55.00–72.00)
Types of statins, n (%)	
Simvastatin	64 (81.01)
Atorvastatin	10 (12.66)
Pitavastatin	5 (6.33)
Doses of statins, n (%)	
2 mg	5 (6.33)
10 mg	40 (50.63)
20 mg	22 (27.85)
40 mg	12 (15.19)
Body mass index (BMI) (kg/m^2)	26.60 (24.50–30.80)
Blood pressure	
Systolic blood pressure (mmHg)	140.00 (126.00–154.00)
Diastolic blood pressure (mmHg)	77.00 (69.00–90.00)
Lipid levels (baseline)	
Total cholesterol (TC), mg/dl	179.00 (156.00–203.00)
LDL cholesterol (LDL-C), mg/dl	104.00 (89.0–124.00)
HDL cholesterol (HDL-C), mg/dl	44.00 (37.00–47.00)
Triglyceride (TG), mg/dl	195.00 (161.00–239.00)
Lipid levels (after 4 months)	
Total cholesterol (TC), mg/dl	170.00 (147.00–200.00)
LDL cholesterol (LDL-C), mg/dl	103.00 (82.00–125.00)
HDL cholesterol (HDL-C), mg/dl	43.00 (37.00–49.00)
Triglyceride (TG), mg/dl	172.00 (140.00–205.00)
Percentage change (%Δ)	
Total cholesterol (TC)	−4.49 (−13.24–4.17)
LDL cholesterol (LDL-C)	−4.81 (−15.31–9.57)
HDL cholesterol (HDL-C)	0.00 (−8.00–5.88)
Triglyceride (TG)	−10.49 (−29.52–11.97)
Comorbidity Hypertension, n (%) Diabetes mellitus, n (%)	23 (29.11) 20 (25.32)

Abbreviation: IQR, interquartile range.

Figure 1A). The percentage change in HDL-cholesterol after 4 months of statin treatment correlated significantly with the *cytochrome P450 7A1 (CYP7A1); −1744G>A* (rs12542233) gene polymorphism (*P*-value <0.01) as shown in Table 3 and Figure 1B. The top significant DMET SNPs associated with the percentage change in total cholesterol after 4 months of

Table 2 Association Between Types and Doses of Statins and Percentage Change in Lipid Profile After 4 Months of Statin Treatment (n=79)

Statin Response	Types and Doses of Statins	Median (IQR)	*P*-value
Percentage change in LDL-cholesterol	Simvastatin 10 mg	−5.13 (−17.14–7.32)	0.56
	Simvastatin 20 mg	−5.26 (−17.09–13.33)	
	Simvastatin 40 mg	−4.81 (−5.91–33.33)	
	Atorvastatin 20 mg	−3.50 (−7.00–0.00)	
	Atorvastatin 40 mg	−5.77 (−12.13–3.61)	
	Pitavastatin 2 mg	7.14 (1.47–40.62)	
Percentage change in HDL-cholesterol	Simvastatin 10 mg	2.27 (−5.56–8.11)	0.84
	Simvastatin 20 mg	−2.13 (−8.16–3.12)	
	Simvastatin 40 mg	−2.13 (−2.27–5.17)	
	Atorvastatin 20 mg	3.85 (−10.71–18.42)	
	Atorvastatin 40 mg	−6.67 (−15.56–4.26)	
	Pitavastatin 2 mg	0.00 (−3.85–14.28)	
Percentage change in total cholesterol	Simvastatin 10 mg	−5.12 (−14.74–2.13)	0.55
	Simvastatin 20 mg	−7.65 (−15.73–4.76)	
	Simvastatin 40 mg	−5.59 (−8.90–25.45)	
	Atorvastatin 20 mg	−6.56 (−9.20–3.92)	
	Atorvastatin 40 mg	−1.50 (−4.91–4.27)	
	Pitavastatin 2 mg	13.79 (2.32–24.70)	
Percentage change in triglyceride	Simvastatin 10 mg	−9.63 (−30.19–18.06)	0.82
	Simvastatin 20 mg	−10.49 (−25.53–9.36)	
	Simvastatin 40 mg	−18.24 (−20.54–11.27)	
	Atorvastatin 20 mg	−21.29 (−39.75–2.83)	
	Atorvastatin 40 mg	11.62 (−21.14–23.44)	
	Pitavastatin 2 mg	0.00 (−14.72–8.70)	

Abbreviation: IQR, interquartile range.

statin treatment were shown in Table 3 and Figure 1C. The analysis showed seven SNPs of three genes (*SLCO1B3*; c.334G > T (rs4149117), c.699A > G (rs7311358), and c.1557G > A (rs2053098), *sulfotransferase family 1E member 1 (SULT1E1)*; c. −64C>T (rs3736599) and c.-9-469A>G (rs3822172), and *ATP Binding Cassette Subfamily B Member 11 (ABCB11); c.-12519C>T* (rs4148768) and c.-8583G>A (rs3770603) were found to be significantly associated with the percentage change in total cholesterol after 4 months of statin treatment (*P*-value <0.01). Among DMET SNPs, the *ATP Binding Cassette Subfamily B Member 4 (ABCB4); c.711A>T* (rs2109505) gene polymorphism presented a significant association with percent change in triglyceride after 4 months of statin treatment (*P* < 0.01) (Table 3 and Figure 1D).

Linkage Disequilibrium Analysis

Linkage disequilibrium analysis of seven SNPs across three genes is shown in Figure 2. Strong linkage disequilibria (D'-values >0.9) were observed between *SLCO1B3* rs4149117, rs7311358, and rs2053098; *ABCB11* rs4148768, and rs3770603; and *SULT1E1* rs3736599 and rs3822172.

Discussion

In the present study, SNPs in drug-metabolizing enzymes and transporter genes associated with lipid-lowering response to statin were identified. The result exhibited that five SNPs; three *SLCO1B3* variants (rs4149117, rs7311358, and rs2053098), one *QPRT* variant (rs13331798), and one *SLC10A2* variant (rs188096) were significantly associated with LDL-cholesterol-lowering response. One SNP of the *CYP7A1* gene (rs12542233) was significantly associated with HDL-cholesterol-lowering response. Seven SNPs; three *SLCO1B3* variants (rs4149117, rs7311358, and rs2053098), two

Table 3 Top SNPs Associated with Percentage Change in Lipid Profile After 4 Months of Statin Treatment (n=79)

Statin Response	SNP rsID	Marker Name	Chromosome	P-values
Percentage change in LDL-cholesterol	rs4149117	*SLCO1B3 c.334G>T*	12	0.0002
	rs7311358	*SLCO1B3 c.699A>G*	12	0.0002
	rs2053098	*SLCO1B3 c.1557G>A*	12	0.0002
	rs13331798	*QPRT_T>G*	16	0.0059
	rs188096	*SLC10A2 c.511G>T*	13	0.0068
Percentage change in HDL-cholesterol	rs12542233	*CYP7A1_-1744G>A*	8	0.0081
Percentage change in total cholesterol	rs4149117	*SLCO1B3 c.334G>T*	12	0.0015
	rs7311358	*SLCO1B3 c.699A>G*	12	0.0015
	rs2053098	*SLCO1B3 c.1557G>A*	12	0.0015
	rs3736599	*SULT1E1 c. −64C>T*	4	0.0095
	rs3822172	*SULT1E1_c.-9-469A>G*	4	0.0068
	rs4148768	*ABCB11_c.-12519C>T*	2	0.0064
	rs3770603	*ABCB11_c.-8583G>A*	2	0.0077
Percentage change in triglyceride	rs2109505	*ABCB4 c.711A>T*	7	0.0064

SULT1E1 variants (rs3736599 and rs3822172), and two *ABCB11* variants (rs4148768 and rs3770603) were significantly associated with the total cholesterol-lowering response. One SNP of the *ABCB4* gene (rs2109505) was significantly associated with triglyceride-lowering response.

Regarding the effect of drug transporter genes, *SLCO1B3* and *SLC10A2* are the hepatic influx transporters that are involved in statins clearance and impact lipid concentration. The *SLCO1B3* gene is translated into organic anion transporting polypeptide 1B3 (OATP1B3) protein, which is the hepatic uptake transporters involved in statins' clearance. In an in vitro study, HeLa cells transfected with *SLCO1B3* (c.334G) and (c.699A) haplotype presented a decrease in statin uptake.[31] Many SNPs of the *SLCO1B3* gene including SNPs 334T>G (Ser112Ala), 699A>G (Met233Ile), 1564C>T (Gly522Cys), and 1564C>T (Gly522Cys) have been analyzed by Letschert et al about functional consequences on protein localization and transport characteristics.[32] A previous study in the Korean population reported that *SLCO1B3* c.334 T>G was associated with higher statin drug concentrations.[33] The present study found *SLCO1B3*-c.334T>G (rs4149117) encoding OATP1B3- S112A, *SLCO1B3*-c.699A>G (rs7311358) encoding OATP1B3- M233I, and *SLCO1B3*-c.1557G>A (rs2053098) encoding OATP1B3- A519A strongly associated with lipid-lowering responses to statin. There are strong linkage disequilibria (D'-values >0.9) among rs4149117, rs7311358, and rs2053098 of the *SLCO1B3* gene. It might be possible that *SLCO1B3* (c.334G > T, c.699A > G, and c.1557G > A) gene polymorphisms may influence statin levels and lipid plasma concentration in the patient treated with a statin. Concerning the *SLC10A2* gene, it is an apical sodium-dependent bile salt transporter (ASBT) or the ileal bile acid transporter which mediates the intestinal absorption of bile salt. As revealed by systematic screening and functional characterization of human *SLC10A2* alleles, the functionally disruptive SNPs are rare.[34,35] The genetic polymorphism of the *SLC10A2* gene is affected by ethnicity. Among genetic variations of the *SLC10A2* gene, the most prevalent was the functionally silent 511G>T (Ala171Ser), found in European American (6%), Chinese American (4.5%), Hispanic American (3.9%), and African American (1.7%) populations.[34] Our study revealed that SNP *SLC10A2*-c.511G>T (rs188096) was significantly associated with the lipid-lowering response to statin. The underlying molecular mechanism of *SLC10A2* gene polymorphisms and lipid-lowering responses to statin should be investigated in further study to reveal the linking molecular pathway.
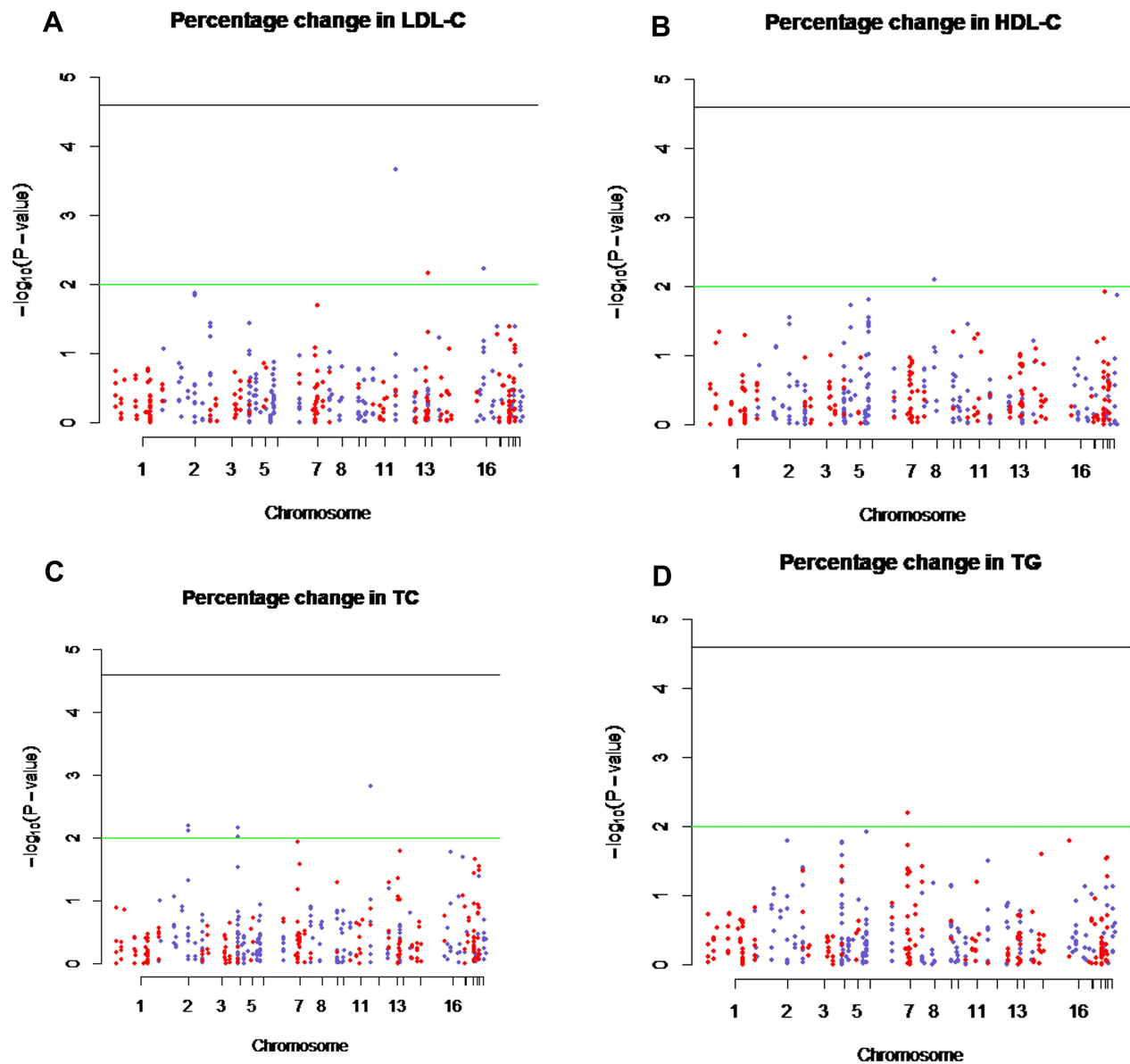
Figure 1 Manhattan plot of – log10 (P-values) from Cochran–Armitage test for trends of 419 single nucleotide polymorphisms (SNPs) in drug-metabolizing enzyme and transporter (DMET) genes and percentage changes in LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), total cholesterol (TC), and triglyceride (TG) levels. The X-axis indicates chromosome number and Y-axis indicates -log10P-values. The green line indicates a P-value of 0.01. The black line indicates a P-value of 2.5×10^{-5}. (**A**) Manhattan plot demonstrates the significant DMET SNPs associated with the percentage change in LDL-cholesterol (**B**) Manhattan plot demonstrates the significant DMET SNPs associated with the percentage change in HDL-cholesterol (**C**) Manhattan plot demonstrates the significant DMET SNPs associated with the percentage change in total cholesterol and (**D**) Manhattan plot demonstrates the significant DMET SNPs associated with the percentage change in triglyceride.

Concerning efflux transporter genes, the present study found that *ABCB4* and *ABCB11* transporter genes polymorphisms were significantly associated with lipid-lowering responses to statin. *ABCB4* and *ABCB11* transporters are both located in the liver and implicated in canalicular bile formation and lipid secretion. *ABCB4* gene, also known as multidrug resistance protein 3 (MDR3), is a membrane-associated transport protein almost exclusively expressed in the liver[36,37] that elaborated in biliary phospholipid secretion.[38] Biliary phospholipids play a key role in solubilizing cholesterol. *ABCB11* gene is translated to the bile salt export pump (BSEP) that is involved in most of the bile salt transport from hepatocytes into the bile canalicular lumen.[39] Hirano et al suggested that BSEP might be elaborated in the biliary excretion of statin in both rats and humans.[40] *ABCB4* and *ABCB11* gene polymorphisms have been reported to affect the composition of bile and were associated with cholestasis and cholelithiasis.[41–43] Hypercholesterolemia is a
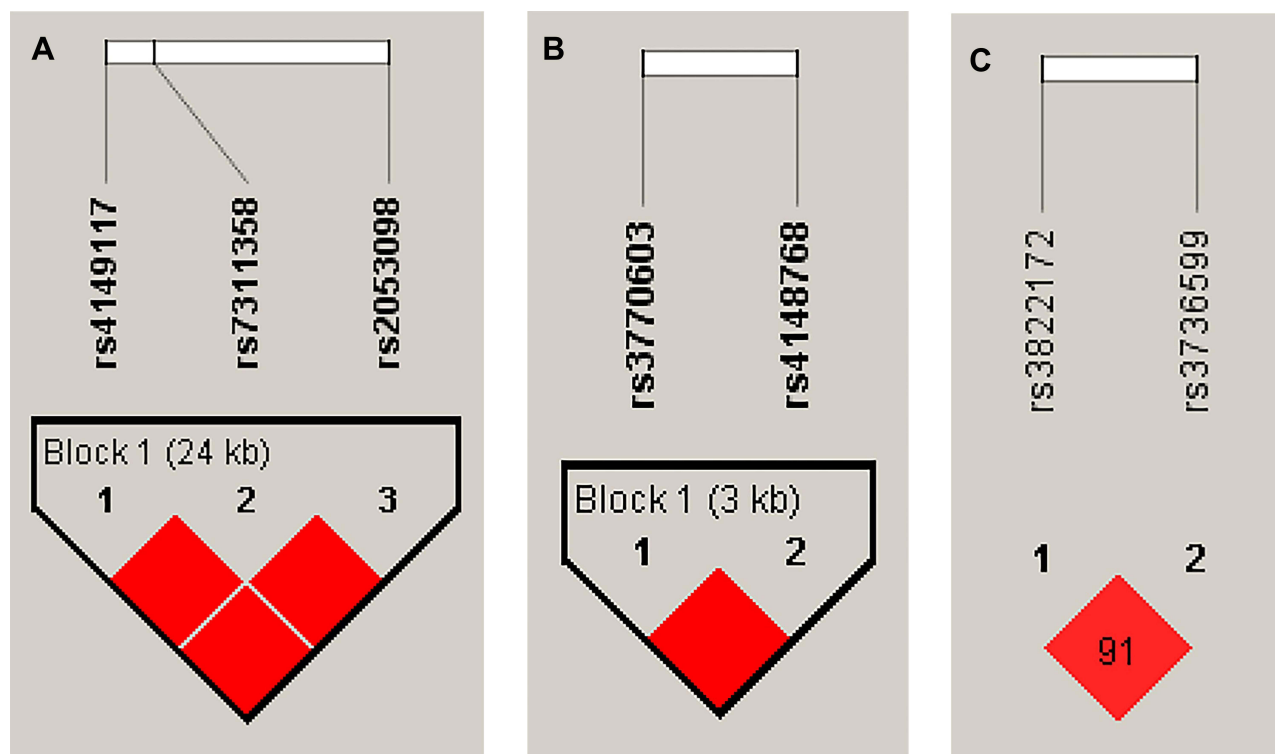
Figure 2 Haploview linkage disequilibrium map of SNPs across DMET genes: (**A**) *SLCO1B3*; (**B**) *ABCB11*; and (**C**) *SULT1E1*. Pairwise linkage disequilibrium (D') values are given in blocks for each SNP combination. Empty dark red blocks indicate D' values of 1.0.

common feature of cholestasis because its metabolic degradation and excretion are impaired. The present study identified one novel polymorphism in *ABCB4* (rs2109505) and two novel polymorphisms in *ABCB11* (rs4148768 and rs3770603) strongly associated with the lipid-lowering response to statin. Taken together with other data, it might be possible that the genetic variations in *ABCB11* and *ABCB4* genes may cause the altered protein function of MDR3 and BSEP leading to alterations in the statins effluxion rate and biliary excretion, which is impact on cholesterol levels.

Cytochrome P450 (CYP) enzyme, cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid production is one of the most promising candidate genes for the determination of individual pharmacological lowering of plasma cholesterol. CYP7A1 is the initial and rate-limiting step for the removal of cholesterol into bile and plays a vital role in maintaining cholesterol homeostasis.[44,45] Many studies have demonstrated that *CYP7A1* variants affect the LDL cholesterol-lowering response to statin.[46–49] A meta-analysis reported that the A-204C (*CYP7A1*) polymorphism was related to the level of total cholesterol and the lipid-lowering efficacy of statin treatment.[50] In this study, we found a significant association between a novel *CYP7A1* polymorphism rs12542233 and reductions of HDL cholesterol response to a statin in Thai patients with dyslipidemia. In further studies, the mechanistic functional effects of *CYP7A1* (rs12542233) polymorphism should be examined to reveal the molecular pathways.

Other top SNPs associated with the lipid-lowering response to statin are the SNP in the *QPRT* gene and SNPs in the *SULT1E1* gene. The QPRT or quinolinate phosphoribosyltransferase is an important enzyme in the synthesis of de novo nicotinamide adenine dinucleotide (NAD+).[51] The increased NAD+ levels were found to stimulate sirtuin 1 (SIRT1) activity, leading to activate the peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α), an important regulator of mitochondrial biogenesis thereby enhancing mitochondrial fatty acid oxidation and reducing triglyceride synthesis. An activated SIRT1 enhances ABCA1 expression by stimulating LXR resulting in increased circulating HDL particles.[52] Moreover, the SIRT1 also reduces SREBP-mediated fatty acid, triglyceride, and cholesterol synthesis by destabilizing SREBPs.[52–54] The study from Shin et al reported that regulation of the key enzymes in the Tryptophan-NAD+ pathway, including QPRT, was associated with PPAR-alpha directly or indirectly.[55] Statins are known to be an activator of PPAR-alpha promoter and mRNA expression.[56] It might be hypothesized that statins may influence

cholesterol levels by indirectly promoting QPRT activity. Our study found that SNP *QPRT*_T>G (rs13331798) was significantly associated with lipid-lowering response to statin. However, this SNP is not in the coding region and does not have direct interactions with lipid-lowering response to statin. Also, the mechanisms underlying *QPRT* gene polymorphisms related to lipid-lowering response to statin has not been elucidated, and further study is needed to discover mechanisms underlying the relationship between the QPRT gene and lipid-lowering response to statin.

Regarding, the *sulfotransferase family 1E member 1* (*SULT1E1*) encoding SULT1E1 enzymes. Sulfotransferases (SULTs) play critical roles in sulfating estrogens, thyroid hormones, bile acids, and other xenobiotics. The previous study showed that SULT1E1 regulates inflammatory response and lipid metabolism by upregulating Peroxisome proliferator-activated receptor-α (PPAR-α),[57] which is a nuclear receptor that regulates lipid and glucose metabolism.[58] Yano et al demonstrated that statins can activate PPAR via the pathway of Cyclooxygenase-2 (COX-2),[59] which is a key enzyme in fatty acid metabolism. Our study found that the novel SNPs *SULT1E1* c. −64C>T (rs3736599) and *SULT1E1*_c.-9-469A>G (rs3822172) associated with lipid-lowering response to statin. Nevertheless, the relationship of SULT1E1 with lipid-lowering response to statin has not been clearly stated in the studies conducted to date. In the future, it is necessary to investigate the mechanistic functional effects of *SULT1E1* gene polymorphism on statin responses to reveal the linking molecular pathways.

In addition to pharmacokinetics genes of statin, the polymorphisms of pharmacodynamics genes such as *CETP, ApoE, lipoprotein lipase (LPL), low-density lipoprotein receptor (LDLR)*, and *LXR* also influence susceptibility to dyslipidemia and statin response.[60–62] Moreover, *LDLR* and *LXR* genes polymorphisms were found to be associated with variations in blood pressure,[63] which is one of the risk factors for cardiovascular disease. Further studies are needed to investigate the impact of the genetic variations in the pharmacodynamic pathway on statin response in the Thai population.

There are several limitations of our study. First, many SNP markers were removed after the QC process because of low minor allele frequency (MAF < 0.05) leading to a missing of the rare genetic variants which may relate to the statin responses. However, selected SNPs (MAF ≥ 0.05) could avoid the false-positive results. Second, the relatively small sample size might have limited the power to examine the relationship between DMET gene polymorphisms and the lipid-lowering response to statins. Third, lack of validation of the significant DMET SNPs and association of lipid-lowering response to statin. The findings should be verified in an independent cohort to avoid the risk of false-positive findings. In addition, some of the SNPs with a suggestive association in this study were not shown to play a vital role in the pharmacokinetic pathway of statin. The evidence of pathway linkup must be established in future studies. Fourth, the changes in serum lipid profile may not be seen clearly due to the short follow-up period. Finally, our study is a retrospective study. The serum lipid profile of the patients at the initiation of statin treatment and other factors involved in statin response such as dietary and lifestyle were not obtainable. Future studies need to design as prospective studies for minimizing the limitations and improving the analysis performance.

Conclusion

In summary, this study genotyped multiple polymorphisms in genes related to phase I and II drug metabolism enzymes and drug transporters and assessed the relationship between DMET SNPs and lipid-lowering response to a statin in the Thai population. Our findings provide a pharmacogenomic approach to further investigate the DMET gene polymorphisms, which impact lipid-lowering response to statin. We suggest further validation of the findings in different cohorts with prospective study design and larger sample sizes. In addition, the evidence of pathway linkup between significant DMET SNPs and lipid-lowering response to statin needs to be established in future studies.

Ethics Statement

This study was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (MURA2019/735). Informed Consent was waived due to the research use specimens leftover from clinical care at the Division of Clinical Chemistry, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. The specimens were not collected specifically for the proposed research, and no additional specimen was collected for this research. Also, the analysis used anonymous clinical data.

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Disclosure

The authors have no conflicts of interest to declare.

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