Genetic and gender factor affect lipid profiles of patients receiving statin treatment in the east coast region of Peninsular Malaysia

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Abstract

Aim: This study was conducted to evaluate the impact of genetic polymorphisms on lipid profiles of statin users. Methods: This retrospective cross-sectional study involved 229 hyperlipidaemic statin users in east coast region of Peninsular Malaysia. DNA was extracted from patients' blood (3mL) and SNP genotyping was performed using PCR-RFLP. Lipid profiles were evaluated before and after statin administration. Multi-factorial impact on the attainment of LDL goal of <2.6mmol/L was assessed by multivariate binary regression. Results: The participants were mostly females (53.3%), Malays (96.1%) and treated with atorvastatin (64.2%). Minor allele frequency (MAF) of the studied SNPs as follow; ABCG2 rs2231142 = 0.12, ABCC2 rs717620= 0.58, APOE rs429358 and rs7412 = 0.35, GATM rs9806699 = 0.63, COQ2 rs4693075= 0.96, and APOA5 rs662799= 0.45. Before statin treatment, ABCG2 rs2231142 (P=0.035) and APOA5 rs662799 (P=0.007) carriers had greater HDL-c levels while ABCC2 rs717620 carriers had higher TC (P=0.040) and LDL-c values (P=0.022). After statin treatment, the following SNPs have affected the lipid profiles; ABCC2 rs717620 (lower TG, P=0.009), APOA5 rs662799 (higher HDL, P=0.031; lower TG, P=0.037) and ABCG2 rs2231142 (higher TC, P=0.038). Furthermore, males were affected lipid profiles significantly than females in APOA5 rs662799 (lower TG, P= 0.038; higher HDL, P= 0.006). Of all independent variables tested, only pravastatin users were predicted patient's achieving LDL-target of <2.6 mmol/L (P=0.040, OR=0.110, 95% CI=0.013-0.902). Conclusion: ABCC2 rs717620, APOA5 rs662799 and ABCG2 rs2231142, as well patient gender, determined different lipid profiles either with or without statin treatment in a subset of Malaysian population.

1.INTRODUCTION

Hyperlipidaemia (HPL) is one of risk factors for cardiovascular diseases (CVD) as previously reported in the Framingham Offspring Cohort.[1] As demonstrated in a meta-analysis of 32 cohort studies conducted in the Asia-Pacific region, HPL was associated with significant increase in CVD mortality with triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were predictors for the CVD risk.[2] Therefore, treatment of HPL is necessary to reduce the prevalence and deaths caused by CVD. Lipids such as low density lipoprotein cholesterol (LDL-c) has been the primary target for lipid reduction in HPL patients. This was evident in a meta-analysis study in the Cholesterol Treatment Trialists' (CTT) Collaborators, demonstrating that 1.0 mmol/L of LDL-c reduction had reduced the coronary mortality by 19.0% (risk ratio=0.81, P <0.0001).[3] Furthermore, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) has highlighted two major modalities in reducing LDL-c, via therapeutic lifestyle changes (TLC) or by lipid-lowering drugs.[4]

One of the most commonly used lipid-lowering drugs, statins, has been identified as the first line of defence against hyperlipidaemia.[5,6] The mechanism of action takes place within the liver, where it competitively inhibits the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, resulting in a decrease in lipid, primarily LDL-c level.[7,8] Statin has been reported to reduce LDL-c levels by 24-60%.[9] The LDL-c lowering effects between individuals, however, vary for a variety of reasons including genetic polymorphisms.[10]

In the era of precision medicine, where more attention is given on the search for genetic predictive markers for treatment efficacy and toxicity, the availability of pharmacogenetic data on statins in Malaysian population is lacking. Statin pharmacogenetic study becomes more crucial to be conducted in a multi-racial country like Malaysia because this will enhance our understanding of the variability of statin effectiveness based on patient factors such as race and gender. Therefore, the current study aims to investigate the association between selected genetic polymorphisms and lipid-lowering efficacy of statins among outpatient statin users in a subset of HPL patients in Malaysia. Based on previous research on gene candidates affecting statin efficacy, the current study originally selected seven single nucleotide polymorphisms (SNP) in six genes related to statin efficacy and toxicity i.e., *CETP* rs708272 (accepted manuscript in the Malaysian Journal of Medical Sciences, MJMS), *ABCG2* rs2231142, *ABCC2*rs717620, *GATM* rs9806699, *COQ2* rs4693075, *APOA5*rs662799 and APOE (rs429358 and rs7412). [11-18]

2.METHODS

2.1 Patient recruitment

This cross-sectional retrospective study involved a total of 229 hyperlipidaemic patients receiving on-going statin treatment from a family medicine clinic at the Hospital Universiti Sains Malaysia (HUSM), one of a major medical centre serving Peninsular Malaysia's East Coast. Patients were recruited between August 2018 and June 2020. Inclusion criteria include: (i) age 18 to 75 years old; (ii) On-going statin treatment for at least six weeks. Exclusion criteria include: (i) being diagnosed with familial hypercholesterolaemia, liver, renal, thyroid or malignant diseases; (ii) taking other medications known to interfere statin efficacy; and (iii) being prescribed with other types of lipid-lowering drugs. All patients' information was gathered from the hospital database including baseline and post-treatment total cholesterol (TC), high density lipoprotein (HDL-c), LDL-c and triglyceride (TG) levels. Current study protocol has been approved by the Human Research Ethics Committee of the hospital (JEPeM) (Reference number: USM/JEPeM/19070437) and in accordance with the Declaration of Helsinki as well as local regulations and standard for ethical review.

2.2 SNP genotyping

Patients who had given their consent were approached, and 3mL of whole blood was collected and stored in EDTA tubes. DNA was extracted according to the manufacturer's protocol (GeneAll Biotechnology, Korea) and stored at -20 until further use. SNP genotyping was performed using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP). The details of primer sequences and specific PCR-RFLP conditions was indicated in Table 1. The PCR steps started with pre-denaturation at 95 for 5 minutes, followed by 35 cycles of denaturation step at 95 for 30 seconds, annealing steps which varied between each SNP (Table 1), and extension step at 72 for 30 seconds. Post-extension was performed at 72 for 10 minutes. To avoid technical errors in genotyping, 5-10% out of total samples were randomly chosen and sent to the Human Identification Unit DNA (HID) at USM, for sequencing analysis.

2.3 Biochemical analysis

Following an overnight fast, blood samples (2 ml) were collected from each participant for lipid assessment (9-12 hours). Biochemical parameters such as TC, TG, HDL-c and LDL-c were analysed using an enzymatic colorimetric method on Hitachi 912 autoanalyser (RANDOX laboratories, United Kingdom) available at the department of Chemical Pathology at the HUSM.

2.4 Sample size calculation

The sample size for the current study was calculated using an online calculator (https://wnarifin.github.io/ssc web.html) and was based on the variant allele frequencies of*CETP* rs708272 (the SNP with the largest sample size as compared to the other studied SNPs), as indicated previously (accepted manuscript in the MJMS). Taking into account the findings of Wanmasae and colleagues in Thailand (a neighbouring country of Malaysia), we considered LDL-c reduction in GG+GA genotypes and AA genotypes as 0.35 (proportion in control, P0) and 0.23 (proportion in case, P1), respectively.[19] Without taking into consideration the dropout

rate, we needed at least 224 participants to achieve the significance level (α) of 0.05 and the statistical power (1-β) at 80%.

2.5 Statistical analysis

SPSS software version 26.0 (IBM, United States) was used to perform statistical analysis. The observed genotype frequencies were checked for deviation from the Hardy-Weinberg equilibrium (HWE) using East Asian (https://asia.ensembl.org/index.html) as the reference population. Continuous data were presented as mean ± standard deviation (SD), and the data's normality was tested using histograms and box plots before being confirmed by the Kolmogorov–Smirnov test. The genetic dominant model was used, and patient genotypes were divided into minor allele carriers (heterozygous + homozygous mutant) and wild-type (homozygous dominant). For normally distributed data, an independent T-test was used to compare lipid levels between two groups, and for non-normally distributed data, a Mann-Whitney U test was used. Lipid levels were compared before and after statin treatment using either one-way repeated measures ANOVA for parametric data or the Friedman test for non-parametric data. A multivariate binary logistic regression analysis was performed to investigate independent factors associated with the likelihood of achieving the LDL-c target of 2.6 mmol/L at the endpoint. P-values less than 0.05 were considered statistically significant.

3.RESULTS

3.1 Characteristics of the patients

Table 2 shows patients' demographic profiles. Patients were 53 ± 7.16 years old on average and female patients slightly higher than their male counterparts (53.3% vs 46.7%). Majority of the patients were Malays (96.1%) and treated with atorvastatin (64.2%), followed by simvastatin (26.2%), pravastatin (7.0%), and lovastatin (2.6%). Diagnosed comorbidities of the patients include diabetes mellitus (DM) and hypertension (HPT) (39.7%), HPT only (37.1%), HPL only (12.7%), and DM and HPL (10.5%), therefore patients were also additional drugs such as antihypertensive and diabetic drugs concurrently. In particular, majority of the patients (38.4%) were prescribed with both diabetic and antihypertensive drugs, 37.1% were on antihypertensive drug, 15.3% were on statin only, and 9.2% were on diabetic drugs. Out of 173 patients of those prescribed with antihypertensive drugs, majority of them were prescribed with two or more combinations of antihypertensive drugs (57.2%), followed by calcium channel blockers (19.1%), angiotensin-converting enzyme inhibitor (13.9%), angiotensin receptor blocker (5.8%), diuretic drugs (2.3%), and β-blocker 1.7 %). In terms of lipid profile, the baseline lipid levels before statin treatment as follow; the mean TC, HDL-c, LDL-c, and TG were 5.72 ± 1.21 mmol/L, 1.30 ± 0.47 mmol/L, 3.72 ± 1.19 mmol/L, and 1.65 ± 0.83 mmol/L, respectively.

3.2 Genotypic and allelic frequencies

Table 3 shows genotypic and allelic frequencies for the current study and comparison with a reference population from the ENSEMBLE website (http://asia.ensembl.org/). The reference population refers to the healthy cohort from East Asian population. For APOE gene haplotype, the genotype frequency of the SNP was compared to a Malaysia's neighbouring country, Thailand.[19] Minor allele frequency (MAF) of the studied SNPs as follow; *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. All SNPs were not in Hardy-Weinberg Equilibrium (HWE) with the reference population (P<0.05) except for *COQ2* rs4693075 ($P = 0.333$).

3.3 The effects of genetic polymorphisms on lipid profiles with and without statins

The effects of the studied genetic polymorphisms on lipid levels of statin users is shown in Table 4. Before statin initiation (baseline levels), certain SNPs associated with different lipid levels. For examples, *ABCG2* rs2231142 was significantly associated with higher HDL-c level (P =0.035); *ABCC2* rs717620 was significantly associated with higher TC (P =0.040) and LDL-c levels (P =0.022); and *APOA5* rs662799 was significantly associated with elevated HDL-c level (P =0.007).

While the effects of statin treatment on lipid levels were observed within two time ranges (i.e., 0-6 months

and 7-12 months) to ascertain the spectrum of the effects right after statin treatment. In all cases, significant association of the dominant genetic testing in two SNPs i.e., *ABCC2* rs717620 (lower TG, P =0.009) and *APOA5*rs662799 (lower TG, P = 0.037) were only observed after 6 months of statin treatment. The dominant effect of SNPs in certain genes (i.e., GATM, APOA5 and APOE) seemed did not predict the lipid lowering effects, especially LDL, since statin treatment themselves resulted in great reductions (P <0.001) of the lipid profiles in both treatment durations. Nonetheless, on the other hand, minor allele carriers of *ABCC2* rs717620 and *COQ2* rs4693075 seemed to predict the statin-related LDL-lowering effects (P <0.001) without significant LDL reduction among homozygous dominant individuals. In all cases, HDL levels were not significantly affected before and after statin treatment.

3.4 Association of other factors in statins affecting lipid profiles

We currently reported that patient factor such as gender, other than determined by the genetic, associated with different lipid profiles (i.e, LDL-c and TG) in hyperlipidaemic patients (n= 229) before statin treatment as in the case with *CETP* rs708272 (accepted manuscript in the MJMS). In particular, higher LDL-c (P =0.007) and TG (P =0.044) were found in females with minor allele A carriers for*CETP* rs708272 (accepted manuscript in the MJMS). Interestingly, although *CETP* rs708272 was no longer resulted in significant LDL level changes in the minor allele A carriers after statin exposure (Table 4), it seems that gender factors did determine the changes of other type of lipid i.e., HDL and TG, especially in males carrying minor allele G of *APOA5* rs662799 (Figure 1a). In particular, minor allele carriers of *APOA5* rs662799 resulted in improved lipid profiles (higher HDL-c, P =0.006; lower TG, P =0.038) in males (Figure 1a), but not in females (data not shown). In contrast, male with *ABCC2* rs717620 (-24C>T) SNP has predicted higher CVD risk since those of CC genotypes had higher TC (P =0.018) and LDL-c (P = 0.008) levels before statin treatment (n= 62, Figure 1b). In the multiple binary logistic regression analysis, only the use of a hydrophilic statin i.e., pravastatin (P =0.040, OR=0.110), but none of the studied SNPs, age and gender factors independently predicted patient's achieving LDL-target of <2.6 mmol/L (Table 5).

4. DISCUSSION

Statins are the first line of defence in the primary and secondary prevention of cardiovascular diseases (CVD) in patients with hyperlipidaemia (HPL).[20] Undeniably, the efficiency of statin treatment against CVD has been reported in numerous clinical studies.[3] The efficacy of statins in improving lipid profiles, could be influenced by several factors including genetic polymorphisms.[21] The current study has expanded on what were evident by previous pharmacogenetic studies related to statin efficacy, and we would like to gain a deeper understanding of how the genetic polymorphisms, along with other patient or clinical factors, could predict statin affecting lipid profiles in a subset of Malaysian HPL patients. For example, when comparing pharmacogenetic data from other continents, Malaysian (a proxy for East Asians) and British (a proxy for Europeans) were related with differing clinical outcomes in other types of genes.[22] Therefore, the current study's findings provide preliminary data on putative genetic variables underpinning inter-individual heterogeneity in statin effectiveness in the Malaysian population, paving the way for future targeted translational research and comparisons with other populations.

Out of the seven SNPs investigated, *ABCC2* rs717620 (C>T) and *APOA5* rs662799 were found to affect lipid profiles in HPL patients with and without statin treatment. Particularly, minor allele T carriers of the SNP experienced significant (P =0.009) TG reduction (Table 4) after at least six months of statin treatment. Therefore, our findings were consistent with a previous study in hypercholesterolaemic Chilean population treated with 10 mg/d atorvastatin (n=127), which showed that minor allele T carriers had a lower TG/HDL index ratio (P =0.030).[23]ABCC2, which encodes the multidrug resistance-associated protein 2 (MRP2) membrane efflux transporter, is essential for cellular efflux of its substrates, including statin, as well as regulating its hepatobiliary excretion.[24] Compared to CC individuals,*ABCC2* rs717620 variants have been linked to lower MRP2 expression and function, resulting in higher bioavailability and, ultimately, efficiency of some types of statins,[24,25] which could explain the TG lowering effect of the SNP in this study.

On the other hand, minor allele T carriers for *ABCC2* rs717620 had lower TC (P =0.040) and LDL-c (P

=0.022) levels in the absence of statin treatment, suggesting a higher risk of HPL among variants (CT and TT genotypes) of the SNP. Since our analysis was not corrected by mean of body mass index (BMI) data of the patients, we could not indicate further the impact of BMI, one of the key confounding factors in influencing lipid levels[26] resulting in high TC and LDL-c levels in the HPL patients before they were treated with statins. A large cross-sectional study in representative samples from the United States (n=12,383) and Spain (n=11,765) found that LDL-c levels were significantly elevated by 23.0 mg/dL and 24.1 mg/dL, respectively, per kg/m^2 increase in BMI (P <0.001 in both populations), though the effect was only observed below the BMI inflection points (27.1kg/m^2 and 26.5kg/m^2, respectively).[27] Similarly, in a non-diabetic Chinese population (n=1,538), the obese group (BMI[?]25kg/m^2) had higher (P<0.01) LDL-c than the lean group (BMI<25kg/m^2).[28]

In terms of TG levels, the current study also found a strong association between *APOA5* rs662799 and TG levels in the presence of statin treatment. APOA5 gene is important in regulating plasma TG levels resulting in TG accumulation in the liver.[11,29]Evidence from both animal and human studies indicated that *APOA5*rs662799 has been linked to a 50% decrease in APOA5 gene expression, which could result in higher plasma TG levels.[30] Also,*APOA5* rs662799 exhibited a significant response in terms of LDL-c reduction (P <0.005) for minor allele G carriers as compared to non-carrier counterpart, following three months of low dose statin treatment in Caucasians (n=154).[31]Consistently, a large cohort study encompassing Hong Kong (n=1,375) and Guangzhou (n=1,996) participants also supported that homozygous recessive GG genotype had 36.1% (P =2.6 x 10^{-13}) and 30.0% (P =1.3 x 10^{-12}) higher plasma TG levels, respectively, than homozygous dominant AA subjects after adjusting for covariates.[32] Two studies in North Iranian (n=199) and Pakistani (n=712) populations reached a similar conclusion: minor allele G carriers for the SNP had a greater risk of elevated TG levels than AA genotypes (OR=1.97, P =0.034 and OR=1.49, P =0.03 respectively).[33,34]

Since *APOA5* rs662799 was associated with higher HDL-c levels (Table 4) at baseline (P=0.007) and to a lesser extent following statin treatment (P=0.031), we postulated that this SNP may have a protective effect against CVD risk, as suggested previously.[35,36,37] In terms of HDL-c levels, our findings are in accordance with those of a study in the Turkish Cypriot population (n=100) which indicated that homozygous recessive GG genotypes had considerably higher HDL-c levels than other genotypes (P =0.014).[38] In addition to higher HDL-c levels, minor allele G carriers also had a lower TG level (P <0.001) after 6 months of statin treatment. To some extent, the findings in this study also reflect a previous finding in multi-ethnic Chinese populations (n=200) where GG genotypes were significantly associated with the greatest TG reduction (P =0.047) than other genotypes (Han ethnicity) following a three-month 20mg/d atorvastatin treatment.[39]

Gender and, to certain degree, ethnicity were two major factors that explained variability in certain lipids such as TG and HDL cholesterol concentrations,[40] thus it is critical to corroborate our results using these two parameters. It is interesting to highlight from our study that *CETP* rs708272 (accepted manuscript in the MJMS), *APOA5* rs662799 and *ABCC2* rs717620 all resulted in gender-specific effects on different lipids. Males with *APOA5*rs662799 polymorphism had higher HDL-c and lower TG levels after statin treatment (Figure 1a) suggesting that the SNP has a protective effect against CVD risk, whereas those with ABCC2 -24C>T variant before statin treatment had higher CVD risk due to higher TC and LDL-c levels (Figure 1b). The gender-specific effect of *APOA5* rs662799 on TG levels in males matched a previous finding in human and mice[41] and to some extent, our findings explained why those with AA genotypes had higher incidence of dyslipidaemia (OR = 1.50, 95%CI, P < 0.001) than their AG and GG counterparts in a large (n= 4,329) longitudinal study.[42] Although we were confident that the*ABCC2* rs717620 was associated with higher TC and LDL-c levels in HPL patients prior to statin treatment (Figure 1b), due to the small number of samples (n=61), our results were limited to the non-significant level for the genotype frequencies of the SNP with the HWE (P >0.055), which may affect the interpretation of our findings. We believe that analysing more samples would increase statistical power and allow us to draw more firm conclusions. In fact, when corrected with Bonferroni adjustment in post-hoc analysis, the significant TC and LCL-c reductions (P <0.001, Table 4) after statin treatment resulted in non-significant associations.

Our study had a few limitations. To begin, the current study solely looked at the effect of SNPs within a single gene on the lipid-lowering efficacy of statins, without taking into account the possibility of gene-gene interaction. For example, the presence of APOE E4 allele in females reduced the effect of *APOA5* rs662799 on TG levels, as demonstrated in a previous study conducted in Caucasians (n=2,500).[43] Likewise, the inclusion of other important genetic predictor in determining statin efficacy, such as solute carrier organic anion transporter family member 1B1 (SLCO1B1) (gene encodes for a membrane-bound sodium-independent organic anion transporter, OATP1B1), the most relevant gene underlying statin-related side effects from a genome-wide association study[44]and has been independently replicated in many gene candidate studies around the world. Clinical factors such as the patient's age and gender, as well as the SLCO1B1 polymorphism, would indeed predict the chance of altered statin pharmacokinetics and likely statin-related myopathy.[45] Secondly, other relevant confounding factors of lipid metabolism need to be considered in the analysis. Factors such as smoking, alcohol intake and BMI could influence lipid parameters and hence had a significant impact on statin response.[46,47] Finally, since the current study's participants were HPL patients and restricted to a single center in Malaysia's east region with the majority of patients in the current study were of Malay ethnicity, the findings must be interpreted with caution when replicated in other ethnic groups in Malaysia or healthy cohorts. The differential response of statin in different ethnicities had also been established in a multi-ethnic nationality such as Singaporean (n=1,589), which concluded that *CETP* rs708272 was associated with HDL-c levels in Chinese males only (P =0.004), but not in other ethnics.[48]

In conclusion, this study found that *APOA5* rs662799 improved lipid profile especially TG and HDL after statin treatment in male, but not female. Minor allele carriers in *CETP* rs708272 and*ABCC2* rs717620 appeared to have higher LDL-c levels prior to statin treatment, but none of the studied SNPs were able to predict whether HPL patients would reach their LDL-target of 2.6 mmol/L after the statin treatment. This finding warrants further research and replication in other Malaysian cohorts with different ethnicities to verify that the observed impact on the lipid profiles was specifically related to Malay-related male statin users.

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COMPETING INTEREST

The authors declare no conflicts of interest

CONTRIBUTORS

N.S.B designed the study, obtained funding and interpreted data analysis of the manuscript. A.F.S drafted the manuscript and contributed to the acquisition, analysis and interpretation of data for the work. A.F.S also involved in patient recruitment and performed the genotyping. N.S.B and A.F.S revised the manuscript and both authors gave final approval and agreed to be accountable for all aspects of the work, ensuring integrity and accuracy.

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FIGURE 1 Gender effect on lipid profiles.docx available at https://authorea.com/users/518812/articles/592834-genetic-and-gender-factor-affect-lipid-profiles-of-patients-receiving-statin-treatment-in-the-east-coast-region-of-peninsular-malaysia