

Variants Near *CETP*, *MTTP* and *BUD13-ZPR1-APOA5* may be Nominally Associated with Poor Statin Response among Filipinos

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ABSTRACT

Objective. Several studies showed that genetic factors affect responsiveness to statins among different populations. This study investigated the associations of candidate genetic variants with poor response to statins among Filipinos.

Methods. In this unmatched case-control study, dyslipidemic participants were grouped into statin responders and poor responders based on the degree of reduction in LDL-c from baseline. DNA from blood samples were genotyped and analyzed. The association of candidate variants with statin response was determined using chi-square and logistic regression analysis.

Results. We included 162 adults on statins (30 poor responders as cases, 132 good responders as controls). The following variants are nominally associated with poor response to statin among Filipinos at a per-comparison error rate of 0.05: rs173539 near *CETP* (OR=3.05, p=0.015), rs1800591 in *MTTP* (OR=3.07, p=0.021), and rs1558861 near the *BUD13-ZPR1-APOA5* region (OR=5.08, p=0.004).

Conclusion. Genetic variants near *CETP*, *MTTP* and the *BUD13-ZPR1-APOA5* region are associated with poor response to statins among Filipinos. Further study is recommended to test the external validity of the study in the general Filipino population.

Keywords: Filipino, *BUD13-ZPR1-APOA5*, *CETP*, lipid, *MTTP*, statin

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INTRODUCTION

Dyslipidemia is a disorder characterized by abnormalities in the lipids circulating in the blood. Around 47.5% of adult Filipinos aged 20 and older have elevated low density lipoprotein cholesterol (LDL-c) levels, with a mean LDL-c level of 131.5 mg/dL (8th National Nutrition Survey - Clinical and Health Survey). Increasing prevalence of elevated levels of LDL-c and triglyceride, and decreased levels of high-density lipoprotein cholesterol (HDL-c) among Filipinos¹ pose a public health concern given their association with the development of atherosclerosis and other cardiovascular conditions. The World Health Organization's Global Health Observatory listed 233,190 total deaths due to cardiovascular diseases in the Philippines in 2016. The latest worldwide estimates show that more than half of the 2 million increase in deaths from 2000–2019 came from the WHO Western Pacific region, to which the Philippines belong. The control of risk factors such as elevated cholesterol levels may delay the development of atherosclerosis and cardiac death.²

Statins have been part of the standard of care since early 1990s as first-line agent for the prevention of coronary artery disease and other cardiovascular events.^{3,4} These drugs act primarily by inhibiting the rate-limiting enzyme HMG-CoA reductase in the synthesis of hepatic cholesterol, thereby upregulating hepatic LDL receptors and reducing the circulating LDL-c pool.

Although proven effective, there is considerable variability in response observed in patients under statin therapy that are not explained by clinical factors. A review demonstrated that Asian patients have better responses to statins compared to Western populations.⁵ Clinical trial evidence show that Asians achieve similar benefits as Westerners at lower statin doses.⁶⁻¹² The potential mechanisms of increased response to statins among Asians are thought to be related to genetically-based differences at the level of hepatic enzymes and drug transporters.

Differences in statin response may be due to genetic polymorphism in *cholesteryl ester transfer protein (CETP)*,¹³ *solute carrier organic anion transporter family 1B1 (SLCO1B1)*,¹⁴ and *proprotein convertase subtilisin-kexin type 9 (PCSK9)*.¹⁵ *CETP* TaqIB polymorphism was studied in over 13,000 patients¹⁶ and has shown to be associated with modified HDL response, where individuals with the B1B1 genotype displayed the greatest HDL-c increases in response to statins in multiple studies.^{17,18} Among Filipinos, the *CETP* TaqIB polymorphism has also been implicated in HDL-c levels.¹⁹ The hepatic uptake of atorvastatin and simvastatin is facilitated by the organic anion-transporting polypeptide 1B1 (OATP1B1), encoded by *SLCO1B1*. Polymorphisms in *SLCO1B1* become potential targets for analyzing impact on statin lipid-lowering response, as these variants could affect OATP1B1 activity. Loss-of-function variants in genes encoding for *PCSK9* have been shown to improve LDL-c reduction with statin use compared to non-carriers

of these variants.²⁰ In addition, a study showed that statins induce *PCSK9* expression, thereby causing dampening of statin-induced LDL-c reduction through LDL receptor degradation.²¹ As a result, various inhibitors to reduce *PCSK9* levels are being studied.¹⁵

Despite the potential significance of genetic influences on statin response, there are no studies that specifically address this concern among Filipinos who remain underrepresented in published literature. This study, therefore, aims to fill this knowledge gap by determining the association of selected genetic variants with poor response to statins in the background of different clinical factors among Filipinos.

MATERIALS AND METHODS

Study design and enrollment of participants

This is an unmatched case-control study investigating the association of candidate variants and clinical factors with poor response to statins among Filipino adults. Participants from a public tertiary university hospital in Manila, different communities in Metro Manila, and private clinics volunteered to be included from July 2013 to March 2017.

We included Filipinos aged 18 years or older with dyslipidemia on statin therapy for a period of at least 16 weeks, if statin-naïve, or at least 6 weeks if previously on moderate intensity statin therapy. Individuals with observed decrease in LDL-c levels of either <50% on high-intensity statin doses or <30% if on moderate-intensity statin doses (Supplementary Table 1) were classified as statin *poor responders* while those with observed decrease in baseline LDL-c levels of either ≥50% if on high-intensity statin doses or ≥30% if on moderate-intensity statin doses were classified as statin *responders*.

Participants were excluded if they had decompensated heart failure, decompensated chronic lung disease, decompensated chronic liver disease, end-stage renal disease, active malignancy, secondary hypertension, secondary dyslipidemia, or pregnancy. They were also excluded if they were related to other enrolled participants up to 3rd degree of consanguinity.^{22,23}

Clinical data collection

We obtained demographic and clinical characteristics of the participants, such as age, sex, co-morbidities, and smoking and alcohol use status from their patient records and from verbal interviews. Previous lipid profile levels prior to use of statins were also recorded from their clinical record. Lipid profile and serum creatinine results were obtained and recorded.

DNA extraction and quantification

DNA was extracted using the QiaAmp DNA Minikit (QIAGEN, Victoria, Australia), following the spin protocol for blood buffy coat specified in the manufacturer's instruction manual. DNA was quantified using a spectrometer at

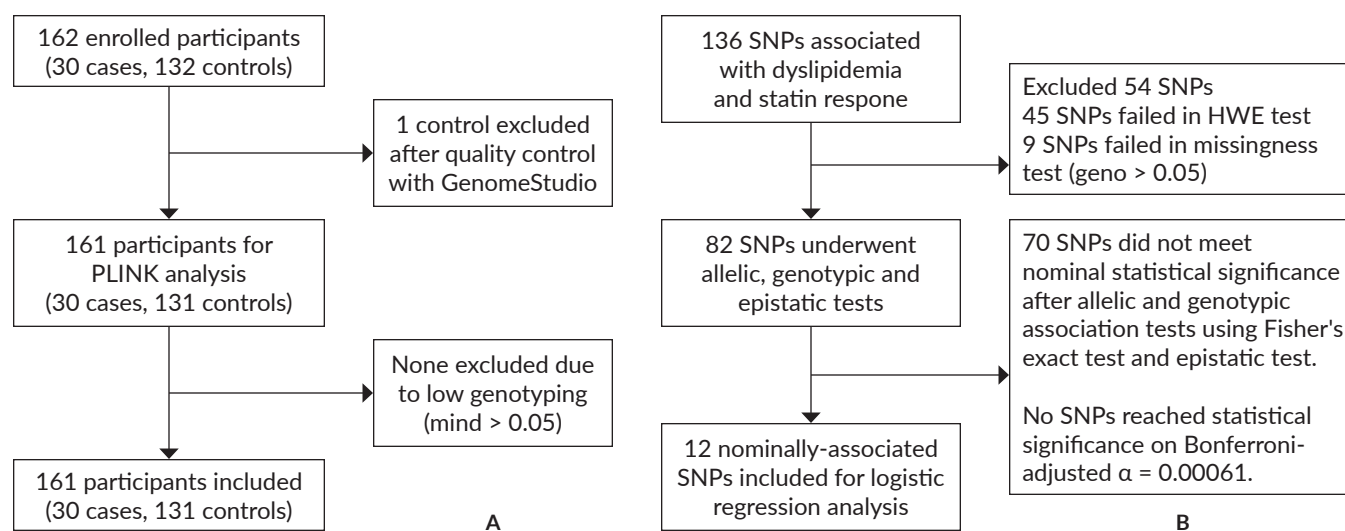


Figure 1. Overview of data processing and analysis. A total of 161 participants (A) and 82 SNPs (B) were analyzed to determine association of clinical metadata and genetic variants with poor response to statins.

SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; geno, genotypic missingness; MAF, minor allele frequency

260nm and stored at -20°C until use. All DNA samples had A-260nm/A-280nm value above 1.80. These were similar to the methods in earlier published studies by the authors.^{22,23}

Genotyping

Researchers from the Microarray Core Laboratory of the Institute of Human Genetics of the National Institutes

of Health-University of the Philippines designed a customized GoldenGate Genotyping (GGGT) BeadChip (Illumina, Inc., San Diego, CA, USA) in 2012 using candidate single nucleotide polymorphism (SNPs) from both coding and noncoding regions, including 136 SNPs which have shown evidence of association with statin response and lipid metabolism (Table 6). These were selected after a

Table 1. Clinical characteristics of study participants

Variable	Poor responders to statin (n=30)	Responders to statin (n=131)	p value
Age \geq 60 years old, %	50.00	52.67	0.79*
Sex, % males	56.67	51.91	0.64*
Co-morbidities, %			
Elevated BMI \geq 25 kg/m ²	50.00	47.69	0.83*
Hypertension	86.67	75.57	0.19*
Type 2 diabetes mellitus	46.67	42.75	0.70*
Ischemic Stroke	20.00	10.69	0.16*
Ischemic Heart Disease	56.67	42.75	0.17*
Smoker, %	40.00	41.54	0.88*
Alcohol use, %	60.00	60.77	0.94*
Laboratory results, mean (SD)			
eGFR, ml/min	74.62 (26.14)	84.77 (26.51)	0.06 [†]
Total cholesterol	202.38 (52.48)	177.35 (48.75)	0.01 [†]
Triglycerides, mg/dl	147.79 (70.26)	125.44 (66.19)	0.10 [†]
HDL-c, mg/dl	48.88 (13.97)	51.52 (15.79)	0.40 [†]
LDL-c, mg/dl	128.03 (39.92)	101.39 (39.96)	0.0012 [†]
Mean percent change in LDL-c level	19.06	53.05	<0.0001 [†]

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein

*Significance set at $p < 0.05$ using chi-squared test

[†]Significance set at $p < 0.05$ using t-test

comprehensive search was done in the following databases: Pharmacogenomics Knowledgebase (PharmGKB) database, National Human Genome Research Institute Genome-Wide Association Study (NHGRI GWAS) Catalog, PubMed and patent databases (e.g., Patentscope and Espacenet) where odds ratios (ORs) for risk and protective effects were provided. While the microarray platform used is optimally designed to detect bi-allelic SNPs, the study included tri- or quad-allelic SNPs in cases where the variants have been correlated with significant clinical outcomes. The selected SNPs were submitted to Illumina, Inc. for scoring to determine the suitability of the SNPs to discriminate genetic variants, as well as estimate their specificity.

Customized genotyping of candidate SNPs was performed using DNA microarray technology following the GGT assay protocol specified in the manufacturer's manual. After microarray processing, the BeadChip was imaged on the HiScan System and data from these images were analyzed using GenomeStudio software. The variant selection and genotyping methods were similar to methods in previously published studies.^{22,23}

Data analyses

Quality control

To ensure high quality data, GenomeStudio 2.0 and PLINK version 2.05.10 were used to identify and remove participants and markers that may lead to false-positive and false-negative associations. Genotype data from participants with call rates >95% after evaluation with GenomeStudio and with individual missingness <0.05 (missingness test) in PLINK were included. The following thresholds were used for the genotype data: minor allele frequency of 0.01 (frequency test), genotype missingness of 0.05 (missingness

test), and Hardy-Weinberg Equilibrium (HWE) of controls of $p < 0.001$ (HWE test).

Statistical analysis

Initial determination of genotypic effect was done. We determined significant differences between alleles and genotypes, i.e., allelic and genotypic association tests (additive, dominant and recessive effects) using Chi-squared and Fisher exact tests were performed to assess for. These were identified based on the distribution of the case and control genotypes. Tests for allelic and genotypic associations were done setting the cut-off at the per-comparison error rate (PCER) of 0.05 to avoid exclusion of SNPs that may be restricted by Bonferroni correction.

Upon determination of genotypic effects, the genotypes were recoded according to the significant effect for logistic regression analysis. Univariate logistic regression analysis was done to determine association of SNPs with poor response to medication, with cut-off p-value set at 0.05. Multiple logistic regression was performed to account for the effects of clinical factors in the odds ratio of the variants (Table 2).

Quality control and statistical analyses were similar to the methods in studies published earlier by the authors.^{22,23}

Ethical considerations

The University of the Philippines Manila-Research Ethics Board reviewed all procedures for compliance with its ethical standards. All participants who volunteered to join the study signed a written informed consent form prior to their interview, chart review and blood extraction. All methods were carried out in accordance with relevant guidelines and regulations.

Table 2. Crude and adjusted ORs of significant clinical and genetic factor

Factors	Crude OR (95% CI)*	Adjusted OR (95% CI)
Hypertension	2.10 (0.68, 6.48)	4.25 (1.08, 16.77)
rs776746 (TC vs TT)	1.70 (0.55, 5.28)	0.88 (0.24, 3.26)
(CC vs TT)	3.10 (1.01, 9.45)	1.77 (0.44, 7.17)
rs2032582 (TT vs TG/GG)	4.09 (1.38, 12.09)	1.60 (0.42, 6.02)
rs1558861 (TC/CC vs TT)	2.89 (1.03, 8.05)	6.98 (0.97, 50.21)
rs10508921 (TC vs TT)	1.44 (0.43, 4.88)	0.99 (0.25, 3.89)
(CC vs TT)	3.54 (1.06, 11.75)	2.24 (0.55, 9.13)
rs651821 (TC/CC vs TT)	3.25 (1.35, 7.84)	1.29 (0.34, 4.88)
rs2075290 (TC/CC vs TT)	2.53 (1.01, 6.31)	0.77 (0.12, 4.72)
rs173539 (CC vs TC/TT)	2.97 (1.31, 6.70)	1.98 (0.67, 5.90)
rs1800591 (GG vs TG/TT)	2.97 (1.23, 7.15)	2.53 (0.88, 7.27)
rs35853021 (TT vs TG/GG)	2.63 (1.09, 6.33)	1.51 (0.52, 4.36)
rs9989419 (GG vs AG/AA)	3.13 (1.39, 7.08)	1.68 (0.60, 4.71)

OR, odds ratio; CI, confidence interval

*variants significant at $p < 0.05$ using logistic regression analysis

Table 3. SNPs with significant association with poor response to statin after variable selection

Variable	Genotypic effect	Adjusted OR (95% CI)	p value*
rs173539	CC vs TC/TT	Recessive	3.05 (1.24, 7.48)
rs1800591	GG vs TG/TT	Recessive	3.07 (1.18, 7.95)
rs1558861	TC/CC vs TT	Dominant	5.08 (1.69, 15.26)

OR, odds ratio; CI, confidence interval

*Significance set at $p < 0.05$ after multiple logistic regression analysis and backward elimination variable selection**Table 4.** The risk allele frequencies of SNPs associated with poor response to statin among Filipinos and other populations

SNP	Reference allele*	Allele frequency for study population	Allele frequencies for other ethnicities [†]					
			Global	AFR	AMR	EAS	EUR	SAS
rs173539	C	0.56	0.67	0.59	0.68	0.77	0.70	0.64
rs1800591	G	0.71	0.75	0.74	0.82	0.86	0.74	0.61
rs1558861	C	0.46	0.13	0.01	0.13	0.24	0.09	0.19

AF, allele frequency; AFR, Africans; AMR, admixed Americans; EAS, East Asians; EUR, Europeans; SAS, South Asians

*The reference allele used among the study population is the risk allele

[†]1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015 Oct;526(7571):68-74.

RESULTS

Thirty poor responders (cases) and 132 responders (controls) were included in the study. There was no difference in sex distribution between the two groups. One control was excluded due to low call rate (Figure 1A). Among the 136 candidate SNPs, 54 were removed based on significant Hardy-Weinberg disequilibrium and genotypic missingness test results. These variants underwent logistic regression analysis to determine the odds ratio.

There was no significant difference observed between statin poor responders and responders in terms of clinical factors (Table 1). The two groups were similar in terms of history of ischemic heart disease (OR 1.75; 95% CI 0.79, 3.90; $p = 0.17$) and stroke (OR 2.09; 95% CI 0.73, 5.98; $p = 0.16$). Most of the participants were on atorvastatin (48%), followed by simvastatin (37%), and rosuvastatin (15%). More than half of the total participants were on moderate-intensity statin therapy (65%). Laboratory results showed a significant difference in the levels of LDL-c and total cholesterol. Higher levels of LDL-c ($p = 0.0012$) and total cholesterol ($p = 0.01$) were observed among the cases, which reflect poor response to statins.

On allelic association analysis, there were 10 SNPs with nominal allelic association with poor response to statin at $\alpha = 0.05$ on simple logistic regression (Table 2). However, none of the variants reached statistically significant association after adjusting for multiple testing (Bonferroni-adjusted $\alpha = 0.00061$). Using Fisher's exact test, rs1800588 and rs662799 were not statistically significant.

On genotypic association analysis, 12 SNPs were found to have nominal genotypic association with poor response to statin at PCER = 0.05 (Table 3). As in the previous step, none of the variants reached statistically significant association after adjustment for multiple testing (Bonferroni-adjusted

$\alpha = 0.00061$) (Figure 1B). Chi-square analysis showed that rs1558861 and rs2075290 were nominally associated with poor response to statins; on Fisher's exact test; however, both SNPs lost statistical significance marginally (0.051 and 0.061, respectively). Both SNPs were retained in the analysis since all cells satisfied assumptions for chi-square test of association. Also, the best possible genotypic effect changed from allelic to dominant for rs1800591 and rs651821 on Fisher's exact test; these were included in further analysis instead.

On univariate logistic regression analysis of clinical factors (Table 4), only hypertension (OR 2.10; CI 0.68, 6.48) demonstrated a p value ≤ 0.2 . Meanwhile, 10 SNPs have been nominally-associated with poor statin response ($p < 0.05$) (Table 5). The crude and the adjusted ORs for these SNPs after multiple regression analysis as adjusted for hypertension are shown on Table 2. Three variants were found with modest association for poor response to statins: rs173539 near *CETP*, rs1800591 in *MTTP* and rs1558861 located in the *BUD13-ZPR1-APOA5* region (Table 3).

DISCUSSION

Genetic factors have been shown to affect responsiveness to statin therapy in different populations. The lack of reliable clinical predictors for poor response to statins highlights the possible utility of pharmacogenetic testing in dyslipidemia management. This study reports on the association of candidate genetic variants to poor response to statins on the background of traditional clinical correlates among Filipinos. The nominal association of three variants are highlighted; namely, rs173539 near *CETP*, rs1800591 in *microsomal triglyceride transfer protein (MTTP)* and rs1558861 located in the *BUD13 homolog-ZPR1 zinc finger-apolipoprotein A5 (BUD13-ZPR1-APOA5)* region.

The variant rs173539 is located 8 kb upstream of *CETP* in the intergenic region. This variant has been implicated in GWAS exploring lipid levels in ancestrally diverse cohorts.²⁴ It has also been linked to the development of metabolic syndrome and cardiovascular disease risk factors in European ancestry individuals.^{25,26} Expression quantitative trait loci (eQTL) analysis published in the Genotype-Tissue Expression (GTEx) Portal demonstrate a possible regulatory function of the variant on *CETP* expression in liver cells. *CETP* expression is shown to increase with every additional C allele.²⁷ This protein is found in plasma, where it is involved in the transfer of cholesteryl ester from HDL-c to very low-density lipoprotein and LDL-c. The inhibition of *CETP* was shown to raise HDL-c levels and decrease LDL-c levels.²⁸ A study by Suhy and colleagues in 2015 showed that the inhibition of *CETP* expression in both liver and spleen is significantly associated with the minor allele T of rs173539, while those with the CC genotype have higher *CETP* expression.²⁹ It is possible that higher *CETP* expression may have caused LDL-c levels to rise, well beyond the statins' threshold for control. Further investigation is needed to verify the possible mechanisms involved.

Another highlighted polymorphism, rs1800591 (-493G>T), is a promoter variant located in the first intron of *MTTP*. Expression studies using HepG2 cells revealed marked enhancement of transcriptional activity with the T allele of the variant.³⁰ The variant forms a haplotype with two other promoter polymorphisms, rs1800804 (-164T>C) and rs1800803 (-400A>T), which determine *MTTP* expression in intestinal and hepatic cells. *MTTP* is necessary for the formation and secretion of lipoproteins containing apolipoprotein B (apoB) from the intestine and liver. Apo B is responsible for the transfer and clearance of LDL-c and has effects on plasma levels.³¹

In this study, participants with the genotype GG of rs1800591 are found to have less LDL-c reductions compared to those with the genotype GT or TT in response to statins. The mechanism behind this association cannot be elucidated in the present study. However, it can be speculated from the roles of *MTTP* and ApoB that an impedance in the clearance of LDL-c can hinder the lowering effect of statins. It has been demonstrated that the T allele of the variant is associated with lower LDL-c serum levels in European³² and Chinese³³ populations. However, there has been conflicting data observed in a cohort of African American³⁴ and Mexican subjects.³⁵ Obesity and hyperinsulinemia likely modulate the effect of the variant on plasma lipoprotein levels and possibly account for the discrepancy.³⁶ Regardless of the variant effect, *MTTP*-inhibitor drugs have been developed which have shown appreciable reductions in LDL-c levels in clinical trials.³⁷

Lastly, rs1558861 is an intergenic variant located down-stream from the region containing *BUD13*, *ZPR1* and *APOA5* on chromosome 11. *BUD13* codes for BUD13 homolog protein, which forms the RES (Retention and

Splicing) complex, together with SNIP and RBMX2. This complex is responsible for splicing regulation and nuclear retention of improperly spliced pre-mRNA.³⁸ *ZPR1* codes for a protein that is essential for normal nucleolar function in proliferating cells.³⁹ *APOA5* codes for an apolipoprotein component of high-density lipoprotein involved in regulating triglyceride levels in the plasma. Other variants in the *BUD13-APOA5-ZPR1* region have been previously associated with attenuated response to statins of LDL-c and triglyceride levels among predominantly-white Americans of Northwestern ancestry, but the mechanisms remain unknown.⁴⁰ The region is also implicated in studies on metabolic syndrome, dyslipidemia and diabetes mellitus, but currently, there are no other studies that directly associate rs1558861 with poor statin response. Meanwhile, an earlier extended genome-wide association study conducted to identify novel genetic determinants of LDL-c, HDL-c and TG levels and their associations in independent populations (including people of Indian Asian descent) has found that the T allele of rs1558861 causes a 0.02-0.04-unit decrease in LDL-c.⁴¹ This is in line with the current study findings wherein allele C of the variant is associated with poorer LDL-c-reduction in response to statins under a dominant model. The allele C frequency among the study population is 46% which is markedly higher compared to other ethnicities (Table 4).

In addition, seven other variants were noted to be associated on univariate analysis which include rs776746 in (*CYP3A5*) and rs2032582 in *ATP binding cassette subfamily B member 1 (ABCB1)*, which are associated with poor statin response among individuals of other ethnicities.⁴²⁻⁴⁵ The variant rs776746 is an allelic splice-acceptor (6986 T>C) causing a decreased or absent *CYP3A5* activity. The expression of this gene varies among different races, which may be indicative of its important role in influencing differences in drug clearance and response.⁴⁶ On the other hand, rs2032582 (2677G > T) is a missense variant in *ABCB1*, a gene involved in multidrug resistance⁴⁷ which include statins and other drugs such as digoxin, chemotherapeutic agents, antiepileptics, and antiretrovirals.⁴⁸

The rest of the variants have previously been found to correspond with lipid parameters across different ethnicities. This includes rs651821 in *APOA5*, rs2075290 in *ZPR1* and rs9989419 near *CETP*.²⁴ The variant rs35853021 in *lipase C, hepatic type (LIPC)* has been associated with HDL-c levels in a Finnish population.⁴⁹ Lastly, the variant rs10508921 in *N-acylsphingosine amidohydrolase 2 (ASAH2)* has been implicated with thiazide-induced changes in triglyceride levels among African Americans.⁵⁰ Although these variants did not retain statistical significance after multiple regression analysis with clinical factors, their notable association with lipid levels and statin response may warrant further investigation.

There are several limitations in this study. While all the drugs used belong to one drug class (statins), subtle

differences in the pharmacokinetics of the varying types of statins may have been an additional confounding variable. Also, given the study design, the study focused on LDL-c reduction as the surrogate outcome measure for more clinically relevant outcomes such as mortality and adverse cardiovascular events, such as myocardial infarction and stroke. As the first study of its kind in the Filipino population, it serves as the springboard for future research that may be validated by prospective study designs, inclusion of clinical endpoints and adverse reactions, larger sample populations, and stratification according to specific subpopulations (e.g., diabetics, post-MI).

At present, guidelines still recommend statins as first-line therapy, with the addition of other drugs only if maximum dose is reached, in the background of suboptimal LDL-c reduction after 4-12 weeks of treatment.³ There are either no recommendations on the use of pharmacogenetic testing to guide statin treatment decision or some groups advise against its use due to lack of conclusive evidence.^{3,4} However, given that poor statin response may lead to further atheroma progression and clinical events,⁵¹ genetic testing has the potential to minimize delays resulting from trial of statin therapy with repeated monitoring of LDL-c levels. Physicians of patients who carry the identified alleles associated with poor response may be guided to immediately intensify or add effective, alternative therapy such as ezetimibe⁵¹ or more recently, PCSK9 inhibitors.^{15,52}

CONCLUSION

Genetic variants rs173539, rs1800591, and rs1558861 are associated with poor response to statins among Filipinos. Further study is recommended to test the external validity of the study in the general Filipino population.

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Author Disclosure

The following authors have no conflicts of interest to declare: Dr. Abrahan, Dr. Agustin, Dr. Aman, Dr. Bejarin, Dr. Cutiongco-de la Paz, Dr. Magno, Dr. Nevado, Dr. Punzalan, Dr. Reganit.

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