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Genetic polymorphism of *CYP3A4* associated with reduced response to statin therapy in Pakistani cardiac and dyslipidemic patients: A cross-sectional observational study

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Abstract: **Background**: Drug resistance phenomenon has become a serious problem in the lipid lowering drugs in the treatment of cardiac and dyslipidemic patients. Statins are the mainstay pharmacological treatment for dyslipidemia, metabolized by *CYP3A4* gene. *CYP3A4* genetic variants are involved in statins resistance, of which *CYP3A4*4* and *CYP3A4*1G* has been observed as important loss of function alleles. **Objectives**: The current study aimed to investigate the *CYP3A4 (CYP3A4*4* and *CYP3A4*1G*) polymorphisms, their association with lipid parameters and influence of variations in response to statin drugs. **Methods**: This cross-sectional observational study included 100 cardiac and dyslipidemic patients receiving statin therapy at Mayo Hospital Lahore, Pakistan. Biochemical assays were performed for lipid parameters and genotyping of *CYP3A4* (*CYP3A4*4* and *CYP3A4*1G)* variants by using allele-specific polymerase chain reaction (AS-PCR) technique. The amplicons of identified variants were electrophoresed and results were verified through Sanger sequencing. **Results**: Serum lipid profile of selected patients presented higher values of total cholesterol (TC) in 51% individuals, total triglycerides (TG) in 79%, LDL- cholesterol in 46% and HDL- cholesterol in 17%. Observed genotypes for *CYP3A4*4* and *CYP3A4*1G* in selected patients included homozygous wild type, homozygous mutant type, heterozygous bi- allelic, as well as tri and tetra- allelic patterns representing multi-locus haplotype combinations. Statistical analysis was performed using SPSS version 27.0. Significant correlations were observed between lipid parameters, whereas *CYP3A4* variants showed modest associations with lipid parameters in studied population. **Conclusion**: The study provides significant inter-racial genetic variations in *CYP3A4*4* and *CYP3A4*1G* in studied population, which might be responsible for the variable response of statins in cardiac and dyslipidemic patients. Although the study addresses the main question about pharmaco-genetic in studied population, the observed genotype–phenotype associations are limited and should be interpreted carefully, emphasizing on the need for additional validation in larger population.

Keywords: Allele; *CYP3A4*; Genetic polymorphisms; Primers; Polymerase chain reaction; Statins

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INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of death worldwide, accounting for an estimated 30% deaths globally (Aliyevich, 2025). Low and middle-income countries, including Pakistan, contribute disproportionately to this burden, where both the incidence and mortality rates are rising steadily. Among the various risk factors, dyslipidemia plays a key role in the pathogenesis of cardiac disorders, being characterized by abnormal levels of lipoproteins such as total cholesterol (TC), total triglycerides (TG), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) (Hedayatnia *et al.,* 2020). Elevated plasma lipid level leads to increase fat deposition in blood vessels especially inside the artery walls, which further starts the cardiovascular complications (Natesan and Kim, 2021). Statins, also known as hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, represent the mainstay pharmacological treatment for dyslipidemia (Iqbal *et al.,* 2018). The load of atherosclerotic cardiovascular disease is significantly reduced by statin treatment (Meiliana and Wijaya, 2023). These agents lower LDL-C and triglycerides, while increasing HDL-C, thereby reducing the risk of atherosclerotic cardiovascular events. Despite their widespread use and proven efficacy, inter-individual variability in response to statin therapy remains a considerable clinical challenge (Patnaik *et al.,* 2022). Some patients achieve optimal lipid control, whereas others show inadequate therapeutic response or develop adverse effects. Failure to achieve expected lipid-lowering response, especially LDL-C levels, in dyslipidemic patients despite the treatment with an optimal dose of statin therapy is considered statin reduced or variable statin response (Bosco *et al.,* 2023). According to "2018 American Heart Association (AHA) guidelines on cholesterol management" patients with atherosclerotic cardiovascular disease (ASCVD) are recommended to receive statins doses to achieve at least a 50% reduction in (LDL-C) levels (Desai *et al.,* 2023).

The cytochrome P450 (CYP) family of enzymes plays a fundamental role in the metabolism of statin drugs (Rajput *et al.,* 2025). Among them, *CYP3A4* is the predominant isoenzyme responsible for metabolizing commonly

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prescribed statins such as simvastatin, atorvastatin and lovastatin (Shuhaili *et al.,* 2017). Genetic polymorphisms within CYP3A4 can significantly alter enzymatic activity, thereby influencing drug metabolism, bioavailability and therapeutic efficacy (Zhang *et al.,* 2024). Single nucleotide polymorphisms (SNPs) are the common form of genetic polymorphism and several SNPs within *CYP3A4* have been linked with inter-individual differences in statin response (Alvarado *et al.,* 2023). *CYP3A4*4*(rs55951658) is a missense variant of *CYP3A4* resulting from a T>C substitution leading to amino acid change *(Ile118Val)* in the *CYP3A4* protein, which may alter the enzyme's activity. *The CYP3A4**1G (rs2242480) variant is an intronic SNP characterized by a G>A substitution also associated with altered *CYP3A4* expression and affecting the metabolism of *CYP3A4* substrates (Saragih and Ichwan, 2025). Such functional alterations may influence statins pharmacokinetics that leads to variability in lipid-lowering response among patients on statin therapy (Nemer and Hendi, 2023).

Prior studies about allelic variants of *CYP3A4* due to genetic polymorphisms in any population, described the critical role of allelic variants in drugs selection and their doses for obtaining optimum results in the field of therapeutics (Sukprasong *et al.,* 2021). According to many past studies, the frequencies of *CYP3A4* genetic polymorphism vary in different ethnicities (Lee *et al.,* 2013). Although genetic polymorphisms in *CYP3A4* enzymes have been extensively investigated in various populations, data regarding *CYP3A4* polymorphisms in relation to statin therapy among Pakistani patients remain scarce. Given the high prevalence of cardiac and dyslipidemic disorders in this population and the widespread use of statins, understanding these genetic variations is crucial for optimizing therapy and moving toward personalized medicine.

Therefore, the present study was designed to investigate the allelic variants of the *CYP3A4* gene in cardiac and dyslipidemic patients receiving statin therapy first time in Pakistani population. By evaluating the lipid profile, amplifying CYP3A4 through allele-specific PCR and validating mutations via Sanger sequencing, this study also provides insight into the genetic basis of statin response variability in an indigenous population. The study specifically aimed to investigate the frequency distribution of CYP3A4*4 and CYP3A4*1G polymorphisms and evaluate their association with lipid profile parameters in Pakistani cardiac and dyslipidemic patients receiving statin therapy.

MATERIALS AND METHODS

Subjects / patients recruitment

This hospital-based cross-sectional observational study was conducted at Mayo Hospital Lahore, Pakistan. All patients for the current study were randomly selected from cardiology ward of Mayo- Hospital Lahore who had been using statins for different time periods; however, the exact duration was not clearly available in the hospital records. The cardiac patients, especially coronary artery disease (CAD) were selected on the basis of angiographic report of patients in hospital record.

Study duration

The study was conducted from June 2024 to December 2024.

Inclusion criteria: (1) Cardiac or dyslipidemic patients on statin therapy; (2) Subjects ages ranged between 18 and 65 years; (3) Patients of either gender (male/ female); (4) Patients signed informed consent for study.

Exclusion criteria: *(*1) Patients with cancer; (2) Pregnant women; (3) Patients with other major co- morbidities; (4) Patients participating in any other clinical study.

Sampling

Sample size was determined using convenient sampling due to limited availability of eligible patients. For each subject, 10 mL of venous fresh blood was collected under fasting conditions (12 hrs.) from cardiology ward of Mayo hospital Lahore, Pakistan. Blood samples were divided into clot-activator tubes (for serum separation) and EDTA vacutainers (for molecular analysis like genomic DNA extraction). Serum was separated from the sample by using a centrifuge at 4,000 rpm for 5 min. and stored at –20 °C until biochemical analysis. Participants with incomplete lab results or demographic data were excluded from analysis.

Biochemical analysis

Serum lipid profile [total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)] was analyzed using standard enzymatic colorimetric methods. All parameters were measured in mg/dL. Assays were performed following the manufacturer's protocols (ABIN2345055 Antibodies Online, Limerick PA, USA) and quality controls were included in each batch.

Molecular analysis

DNA extraction: DNA extraction was performed through "Genomic DNA Extraction kit (GeneJET™ Genomic DNA Purification Kit Thermo Fisher Scientific, Lithuania)". To evaluate the DNA quality agarose gel electrophoresis technique was used. The quantification of DNA was done with a spectrophotometer (NanoDrop™, Thermo Scientific, USA).

Primer designing: Primers (allele specific) for *CYP3A4*4* as well as *CYP3A4*1G* were designed through Primer 3 (http://primer3.ut.ee). Primers (allele specific) were also used for amplification of genomic DNA flanking the SNP.

Table 1: *CYP3A4*4* (rs55951658) genotype primers

CYP3A4 allele	Name of primer	Sequence of primer	Optimized annealing temperature (forward/reverse) (˚C)
*CYP3A4*4*	C forward	5'CACATTTTCTACAACCATGGAGACC 3'	59
	G forward	5'CACATTTTCTACAACCATGGAGACG 3'	59
	A forward	5'CACATTTTCTACAACCATGGAGACA 3'	57.5
	T forward	5' CACATTTTCTACAACCATGGAGACT 3'	57.5
	Universal reverse	5'TTTATACCTGTCCCCACCAGATTC3'	

Table 2: *CYP3A4*1G* (rs2242480) genotype primers

CYP3A4 Allele	Name of primer	Sequence of primer	Optimized annealing temperature (forward/reverse) (˚C)
*CYP3A4*1G*	A forward	5' TCACCCTGATGTCCAGCAGAA 3'	56.5
	C forward	5' TCACCCTGATGTCCAGCAGAC 3'	57
	T forward	5' TCACCCTGATGTCCAGCAGAT 3'	56.5
	G forward	5' TCACCCTGATGTCCAGCAGAG 3'	57
	Universal reverse	5'GTAATAGAAAGCAGATGAACC3'	

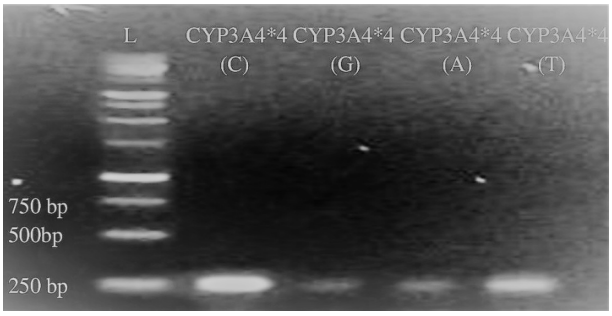

Fig. 1A

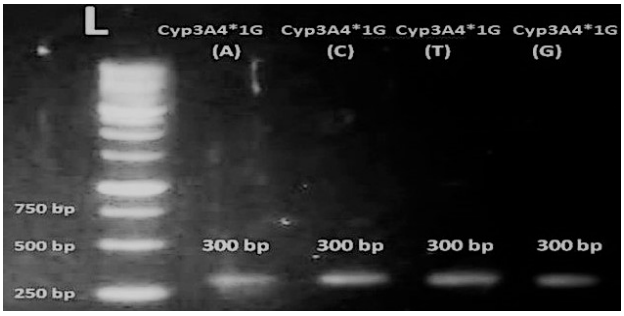

Fig. 1B

Fig. 1: Gel is showing the allele-specific PCR product of (Fig 1A) *CYP3A4*4* (SNPs C, G, A and T). *CYP3A4*4*, **4* allele of cytochrome P450 3A4; bp, base pairs; L, NovelGene 1kb DNA ladder RTU; (Fig 1B) *CYP3A4*1G* (SNPs A, C, T and G). *CYP3A4*1G*, **1G* allele of cytochrome P450 3A4; bp, base pairs; L, NovelGene 1kb DNA ladder RTU.

Four pairs of primers were used for amplification, one pair with wild- type primer and remaining three with mutant type allele specific primer; non- allele specific reverse primer was common for both genotypes. To design the allele specific primers the strategy followed as of Hirotsu *et al.,* (2010). Primers detail is described in table 1 and 2. To minimize selection and measurement bias, standardized biochemical assays and validated AS-PCR procedures were used.

PCR amplification: PCR was performed in 25 µL reaction volume containing 13 µL master mix (Thermo Fisher Scientific), 4 µL genomic DNA, 2 µL of each oligo-nucleotide (forward and reverse) primers and 4 µL sterile water.

The conditions used for PCR were; 5 min at 95 ˚C for each cycle, 35 cycles at 95 ˚C for 30 sec, with specific annealing temperatures as discussed in (Table 1 and 2) for 30 sec and 72 ˚C for 30 sec, followed by 1 cycle at 72 ˚C for 7 min. MiniAMP Plus Thermal Cycler (Applied Biosystems™, Thermo Scientific) used for PCR. Moreover, 10 samples for each allele were randomly selected to assess repeatability. Negative control included for AS-PCR procedure each time. No errors were detected during the AS-PCR procedure.

Gel electrophoresis: Gel electrophoresis of amplified SNP products were performed on 1.5% agarose gel stained with ethidium bromide (EtBr). UV Trans illuminator (Benchtop 3UV Trans illuminator; UVP, USA) used for visualizing the outcomes of gel electrophoresis. Allele-specific bands were recorded and compared with NovelGene (www.novelgene.com) 1kb DNA ladder RTU. The *CYP3A4* variant (*CYP3A4*4* and *CYP3A4*1G)* were genotyped through gel- based genotyping technique Figs. 1A and 1B.

Sanger sequencing: Sanger sequencing of sixteen (16%) randomly selected samples, eight from each *CYP3A4*4* and *CYP3A4*1G* allelic variants (wild types and mutant type), was performed to confirm the results of gel- based

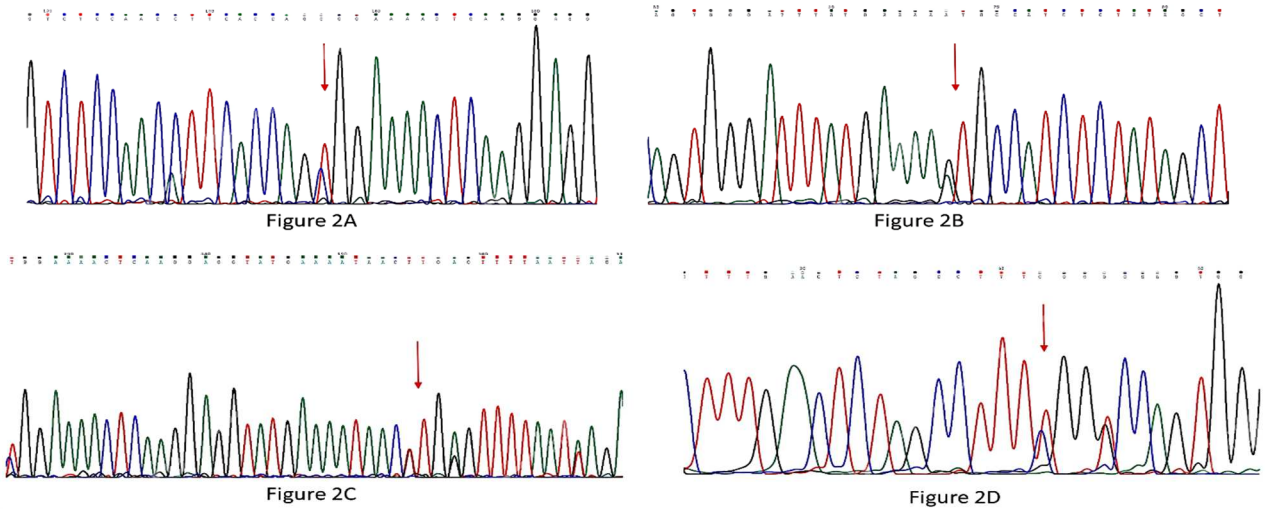

Fig. 2: Sequencing chromatograms of (forward primer used for sequencing) (A) CYP3A4*4C; (B) CYP3A4*4G; (C) CYP3A4*4A; (D) CYP3A4*4T.

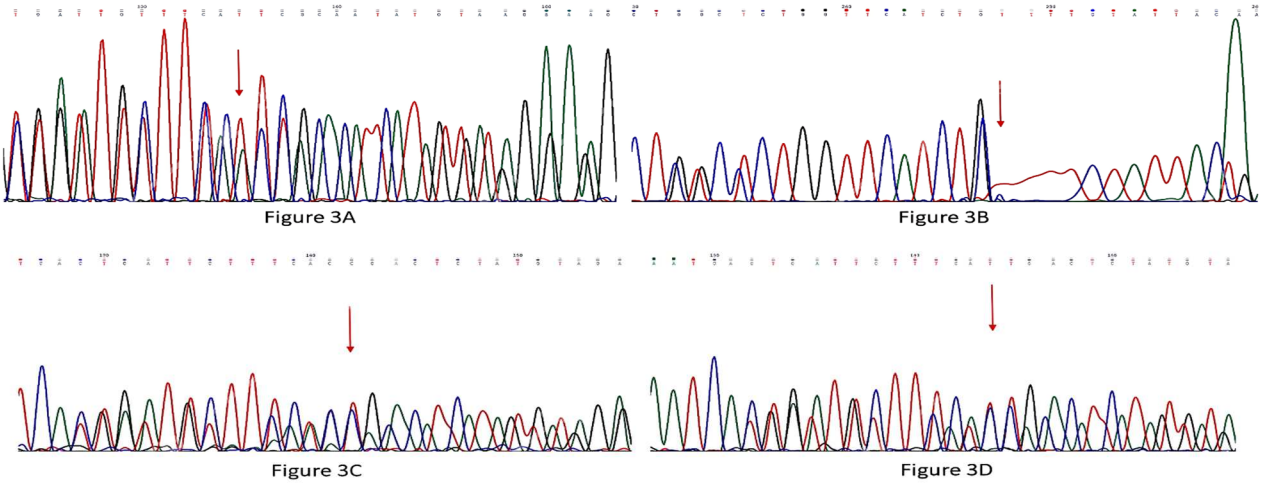

Fig. 3: Sequencing chromatograms of (forward primer used for sequencing): (A) CYP3A4*1GA; (B) CYP3A4*1GC; (C) CYP3A4*1GT; (D) CYP3A4*1GG

technique for SNP's identification of *CYP3A4*4* and *CYP3A4*1G.* Sequencing of purified PCR products were performed by using forward primers. Selected PCR amplicons were purified and subjected to Sanger sequencing commercially outsourced (Macrogen Inc., Seoul, South Korea). Chromatograms of Sanger sequencing were analyzed with Chromas software (Technelysium Pty Ltd., South Brisbane, Australia) to validate SNPs identified by allele-specific PCR.

Statistical analysis

Data were analyzed by SPSS software (27.0; IBM Corp., USA). Shapiro–Wilk test was performed to check the normality of data distribution. Descriptive statistics used to analyze the frequencies of gender, age and lipid profile. Pearson correlation test used to check the relationships between lipid profile parameters. Additionally, Linear Regression model was used to evaluate the association between genotype and lipid profile. P-values < 0.05 and < 0.01 were considered statistically significant.

RESULTS

The current study screened Pakistani cardiac and dyslipidemic patients receiving statin therapy for the reduced function alleles *CYP3A4*4* and *CYP3A4*1G*, which are involved in variable statins responses among different racial groups. In this study, a sum of 100 cardiac and dyslipidemic patients of both male (52) and female (48) on statin therapy were analyzed for serum lipid profile and also through allele specific PCR (AS- PCR) identification of single nucleotide polymorphism (SNP). Among screened patients, 100 eligible participants

fulfilling inclusion criteria were included in the final analysis. The age range of subjects was 18-65 years, with the mean age of male and female 40.94 ± 9.84 and 44.66 ± 11.97 respectively. Table 3 demonstrates the summary of subjects' gender and age.

Table 3: Summary of Subjects' Gender and Age

Total subjects/patients	100 (n=100)
Total male	52%
Total female	48%
Mean age (yrs) male	40.94 ± 9.84
Mean age (yrs) female	44.66 ± 11.97

Biochemical analysis

The results of serum lipid profile of selected individuals showed that 51% of them were higher in total cholesterol level, 79% in triglycerides (TG) level, 46% in LDL-cholesterol and 17% in HDL cholesterol. The results also showed that the HDL cholesterol was lower in 10% selected patients. Table 4 describes the descriptive statistics of lipid profile in selected patients.

PCR and Sanger sequencing

The amplified products of allele specific PCR (AS-PCR) for four allelic variants of *CYP3A4*4* and *CYP3A4*1G* were confirmed through Sanger sequencing method. The sequencing chromatograms results aligned 100% with the results of the gel electrophoresis (Fig. 2 and Fig. 3).

Allele frequency distribution

The allele frequency distribution of *CYP3A4*4* and *CYP3A4*1G* and their allelic variants were first time calculated in cardiac and dyslipidemic patients of Pakistani population. Among those 100 cardiac and dyslipidemic patients which were analyzed for allelic variants of *CYP3A4*4* in current study, 50% patients were heterozygous for *CYP3A4*4 C/T* bi-allelic variants, 2% for *G/A/T* tri-allelic variants, 3% for *C/G* bi-allelic variants, 1% for *A/T* bi-allelic variants, 8% for *C/G/T* tri-allelic variants, 3% for *C/G/A/T* tetra allelic. Whereas, 18% variants were homozygous for *C* wild type variant, 7% for *T* mutant type, 1% for *G* mutant type and 1% for *A* mutant type. There were 6% patients from selected population which did not show any allelic variant of *CYP3A4*4* rather have wild type genotype of *CYP3A4*. Table 5 describes the frequency distribution of *CYP3A4*4* allelic variants in studied population.

On the other side the allelic variants of *CYP3A4*1G*, 35% patients were heterozygous for *A/C/T/G* tetra allelic variants, 10% for *A/C/G* tri-allelic, 2% for *A/T/G* tri-allelic, 4% for *C/T/G* tri-allelic and 3% for *A/C/T* tri-allelic variants. The bi-allelic variants were 8% for *A/T*, 4% for *A/C*, 1% for *A/G*, 1% for *C/T* and 9% for *T/G*. Whereas, 8% were homozygous for mutant type *A* variant, 3% for mutant type *C* variant and 10% for *G* wild-type. There were 2% patients from selected population which did not show any allelic variant of *CYP3A4*1G* rather has wild type genotype of *CYP3A4*. Table 6 describes the frequency distribution of *CYP3A4*1G* allelic variants in studied population.

Tetra-allelic (multi-locus SNP combinations) SNPs in the *CYP3A4*4* (*CYP3A4*4 C/G/A/T*) and *CYP3A4*1G* allele (*CYP3A4*1G A/C/T/G*) were first time identified in Pakistani dyslipidemic patients. To the best of our knowledge, no evidence found in the literature about tetra- allelic (multi-locus SNP combinations) variants of *CYP3A4*4* and *CYP3A4*1G* in any other populations, whereas, bi and tri- allelic (multi-locus SNP combinations) variants have been observed in many other populations.

Correlations

Between lipid parameters

The Pearson correlation analysis was performed to examine the association among lipid parameters. Total cholesterol (TC) showed a strong positive correlation with triglycerides ($r = 0.747$, $p < 0.001$) and LDL-C ($r = 0.947$, $p < 0.001$) and a moderate positive correlation with HDL-C ($r = 0.353$, $p < 0.001$). Triglycerides also showed positive association with LDL-C ($r = 0.662$, $p < 0.001$), whereas a weak but significant negative correlation was observed between triglycerides and HDL-C ($r = -0.223$, $p = 0.026$). Furthermore, LDL-C demonstrated a moderate association with HDL-C ($r = 0.300$, $p = 0.002$). Overall, in current study, lipid parameters indicated significant correlations among each other. Table7 describes the Pearson correlation analysis of lipid profile in selected patients.

Between genotype and lipid parameters

The linear regression analysis was performed to check the association among genotypes and lipid parameters. According to the ANOVA results of linear regression analysis the *CYP3A4*4* showed weak but statistically significant positive correlation ($\rho = 0.218$, $P < 0.05$) with total cholesterol, whereas, no significant association was observed with triglyceride ($\rho = 0.042$, ($P > 0.05$) and LDL-C levels ($\rho = 0.127$, ($P > 0.05$). However, *CYP3A4*4* and HDL –C levels indicated significant positive relation ($r = 0.638$, $P < 0.001$).

On the other hand, the ANOVA results of linear regression analysis showed no significant association between *CYP3A4*1G* and total cholesterol or triglyceride levels ($P > 0.05$). However, not significant ($P > 0.05$) negative correlation observed in case of *CYP3A4*1G* and LDL-C levels. *CYP3A4*1G* and HDL-C levels showed statistically significant ($P < 0.001$) correlation. Table 8 and 9 showed the ANOVA results of linear regression analysis between genotype and lipid profile in selected patients.

Table 4: Descriptive statistics lipid profile results in selected patients.

	N	Minimum	Maximum	Mean	Std. deviation
TG	100	9.00	369.00	192.9400	51.85544
TC	100	68.00	1116.00	245.4200	142.73647
LDL	100	22.00	254.00	120.0000	56.57265
HDL	100	13.00	281.00	64.6500	47.19172
Valid N (list wise)	100				

Table 5: Allelic variants of *CYP3A4*4* (rs55951658) in Pakistani cardiac and dyslipidemic patients.

Genotype	Non-allelic variants percentage %	Allelic variants percentage %									
		Homozygous				Heterozygous					
		C/C wild type	*T/T mutant type*	*G/G mutant type*	*A/A mutant type*	*C/T Bi - allelic*	*C/G Bi - allelic*	*A/T Bi - allelic*	*G/A/T Tri - allelic*	*C/G/T Tri - allelic*	*C/G/A/T Tetra-allelic*
*CYP3A4*4*	06%	18%	07%	01%	01%	50%	03%	01%	02%	08%	03%

Table 6: Allelic variants of *CYP3A4*1G* (rs2242480) in Pakistani cardiac and dyslipidemic patients.

Genotype	Non-allelic variant percentage %	Allelic variants percentage %		
*CYP3A4*1G*	02%	Homozygous	*G/G wild type*	10%
			C/C mutant type	03%
			A/A mutant type	08%
		Heterozygous	*A/T Bi -Allelic*	08%
			A/C Bi -Allelic	04%
			A/G Bi -Allelic	01%
			C/T Bi -Allelic	01%
			T/G Bi -Allelic	09%
			A/C/G Tri -Allelic	10%
			A/T/G Tri -Allelic	02%
			C/T/G Tri -Allelic	04%
			A/C/T Tri –Allelic	03%
			A/C/T/G Tetra-Allelic	35%

Table 7: Pearson correlation analysis of lipid parameters

		TC	TG	LDL	HDL
TC	Pearson correlation	1	.747**	.947**	.353**
	Sig. (2 tailed)		.000	.000	.000
	N	100	100	100	100
TG	Pearson correlation	.747**	1	.662**	.223*
	Sig. (2-tailed)	.000		.000	.026
	N	100	100	100	100
LDL	Pearson correlation	.947**	.662**	1	.300**
	Sig. (2-tailed)	.000	.000		.002
	N	100	100	100	100
HDL	Pearson correlation	.353**	-.223*	.300**	1
	Sig. (2-tailed)	.000	.026	.002	
	N	100	100	100	100

TC (Total Cholesterol); TG (Triglycerides) **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 8: ANOVA results of linear regression analysis between *CYP3A4*4* and lipid profile.

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	12715.048	1	12715.048	23.984	.000[b]
Residual	48772.697	92	530.138		
Total	61487.745	93			

a. Dependent Variable: Cholesterol
b. Predictors: (Constant), *CYP3A4*4*

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	338597.037	1	338597.037	12.181	.001[b]
Residual	2557362.931	92	27797.423		
Total	2895959.968	93			

a. Dependent Variable: Triglycerides
b. Predictors: (Constant), *CYP3A4*4*

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	1555.717	1	1555.717	5.896	.017[b]
Residual	24275.996	92	263.870		
Total	25831.713	93			

a. Dependent Variable: LDL-C
b. Predictors: (Constant), *CYP3A4*4*

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	165.699	1	165.699	10.726	.001[b]
Residual	1421.237	92	15.448		
Total	1586.936	93			

a. Dependent Variable: HDL-C
b. Predictors: (Constant), *CYP3A4*4*

DISCUSSION

A sum of 100 subjects was included in the current study. The mean total cholesterol level was 192.94 ± 51.86 mg/dL, (range: 9 - 369 mg/dL). Mean triglyceride level was 245.42 ± 142.74 mg/dL (range: 68–1116 mg/dL).

Mean LDL-C level was 120.00 ± 56.57 mg/dL, (range: 22 - 254 mg/dL) and mean HDL-C level was 64.65 ± 47.19 mg/dL (range: 13- 281 mg/dL). The findings should be interpreted cautiously due to the relatively small sample size and absence of baseline lipid measurements before statin therapy.

The Shapiro–Wilk test confirmed that only age followed a normal distribution, however total cholesterol ($P< 0.05$) and triglycerides ($P< 0.001$), LDL-C ($P< 0.05$) and HDL-C ($p < 0.001$) were not normally distributed. The strong positive association between total cholesterol and LDL-C was observed, while, negative association between triglycerides and HDL-C further reflects the typical dyslipidemic pattern observed in patients with cardiovascular risk. The linear regression analysis showed a weak but significant positive relation between the *CYP3A4**4 genotype and total cholesterol levels, while no significant relationships were observed with triglycerides or LDL-C. A strong positive correlation between *CYP3A4**4 and HDL-C was observed, suggesting a potential influence of this variant on HDL-C metabolism. However, *CYP3A4**1G showed no significant association with total cholesterol, triglycerides, or LDL-C, while a moderate correlation with HDL-C was detected. Overall, these outcomes suggest that the examined *CYP3A4* polymorphisms may have a limited influence on most lipid parameters in the studied population. “Tri-allelic” and “tetra-allelic” terms are used to describe the “multi-locus SNP combinations” within the amplified region of *CYP3A4*. Multi-locus SNP combinations observed due to the presence of multiple polymorphic sites occurring simultaneously in individual samples. To overcome the concern about possible laboratory artifacts: genotyping was performed using allele-specific PCR; randomly selected samples were independently validated through Sanger sequencing to confirm the presence of the detected variants.

Table 9: ANOVA results of linear regression analysis between *CYP3A4*1G* and lipid profile.

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	567.671	1	567.671	.709	.402[b]
Residual	78472.969	98	800.745		
Total	79040.640	99			

a. Dependent Variable: Cholesterol
b. Predictors: (Constant), *CYP3A4*1G*

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	163404.033	1	163404.033	5.805	.018b
Residual	2758350.477	98	28146.433		
Total	2921754.510	99			

a. Dependent Variable: Triglycerides
b. Predictors: (Constant), *CYP3A4*1G*

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	519.251	1	519.251	1.407	.238b
Residual	36173.499	98	369.117		
Total	36692.750	99			

a. Dependent Variable: LDL-C
b. Predictors: (Constant), *CYP3A4*1G*

ANOVA[a]					
Model	Sum of squares	df	Mean square	F	Sig.
Regression	3.175	1	3.175	.146	.704b
Residual	2135.825	98	21.794		
Total	2139.000	99			

a. Dependent Variable: HDL-C
b. Predictors: (Constant), *CYP3A4*1G*

These combined variants form haplotypes that could collectively influence *CYP3A4* enzymatic activity and lipid metabolism (Wang *et al.,* 2016). Structural instability due to base mismatches could introduce mutational changes in both strands of DNA duplex. For example, a substitution of a G–C base pair to G–A may create a mismatched intermediate that increases susceptibility to further mutation, potentially resulting in a C–A pairing.

If DNA replication process continues through such mismatched bases without repair, the original G allele may eventually be transformed into both C and T variants in subsequent replication cycles. On the other hand, simultaneous mutations could occur on complementary DNA strands due to external mutagenic influences such as chemical or radiation exposure. Even though most (SNPs) in the human genome are bi-allelic, but tri-allelic and, rarely, tetra-allelic sites have also been documented. The frequency of tri-allelic sites has been reported to be approximately twice that expected under random mutational models. Proposed mechanisms to explain this excess: (1) the presence of hyper mutable regions in DNA; (2) the simultaneous generation of two alternative alleles at a single site within an individual; and (3) During hetero duplex formation of recombination events, arising of secondary mutations from base mismatching Mutation rates are strongly influenced by local sequence framework; a well-known example is the CpG dinucleotide, which shows increased rates of both types of mutations including transition and transversion. Other neighboring nucleotide sequences might modulate mutational susceptibility (Rehman *et al.,* 2015).

Based on these results, the study provides novel insights into the distribution of *CYP3A4* alleles and identifies multi-locus SNP combinations in Pakistani cardiac and dyslipidemic patients, a population largely underrepresented in pharmacogenetic research. The observed population-specific patterns, along with correlations between genotype and lipid profiles, may guide clinicians in optimizing statin therapy in indigenous population. Numerous previous studies have demonstrated that genetic polymorphisms in the studied gene influence statin metabolism.

Racial differences significantly contribute to variability in *CYP3A4* activity, which may influence the metabolism of drugs their therapeutic response and the risk of drug toxicity. Interracial variation in *CYP* enzyme activity has been broadly documented and is often recognized to alterations in genetic polymorphisms, physiological and nutritional factors (Saragih *et al.,* 2025). As shown in present study, the significant difference among racial and ethnic groups found in *CYP3A4*4* and *CYP3A4*1G*. The allelic frequencies of *CYP3A4*4* in studied population shown that heterozygous variants were very high as compared with other studied populations, such as, 3.32% reported in Chinese hyperlipidemic patients (Wang *et al.,* 2005) and 0.18% in Chinese Han population (Hu *et al.,* 2017).

In African population the estimated *CYP3A4*4* frequency is around 0.3% (Alessandrini *et al.,* 2013). On the other side, allele frequency distribution of *CYP3A4*1G* (20230G>A) is higher in East Asian population around 15-30%. *CYP3A4*1G* is also the most common variant in Chinese Han population, as reported 24.01% (Hu *et al.,* 2017) and 53% in Indonesian population (Atmaja *et al.,* 2024), 18.8% in Chinese Han Population (He *et al.,* 2011). In Native American population the frequency is around 27% which is also on higher side, 29.7% in Japanese population and 88.9% in Yoruba in Ibadan, Nigeria (Fohner *et al.,* 2013).

Genetic polymorphisms in the *CYP3A4* variants may influence the metabolism and therapeutic response of multiple statins, with some (*CYP3A4*4*, *CYP3A4*1G, CYP3A4*1B* and *CYP3A4*3*) showing well-documented effects, while others require further validation (Nemer *et al.,* 2023). The differences in the frequencies of *CYP3A4*4* and *CYP3A4*1G* and their allelic variants are accountable for the statins drug response in cardiac and dyslipidemic patients treatment (Lusiki *et al.,* 2024). As these SNPs are responsible for reduce drugs activities like *CYP3A4*4* SNP associated with reduce enzyme activity of *CYP3A4* leading to reduce simvastatin activity towards its lipid lowering effects in hyperlipidemic patients (Wang *et al.,* 2005). According to (He *et al.,* 2014) the bioavailability of atorvastatin (orally administered) was reduced in coronary heart disease patients of Chinese Han population with *CYP3A41G/1G* genotype, resultantly, associated with decreased cholesterol- lowering effectiveness of atorvastatin. On the other side (Gao *et al*., 2008) observed that the *CYP3A4*1G* allele enhance the efficacy of atorvastatin. The reason of this may be due to other variations like *SLCO1B1 521* T>C and *CYP3A5*3* which are influencing the pharmacokinetics of atorvastatin along with the efficacy, were not expelled by Gao *et al.,* 2008 (He *et al.,* 2014). (Kadam *et al.,* 2016) also observed the low activity of atorvastatin in *CYP3A4* variants as compared to wild type. In Egyptian population disturbance in atorvastatin pharmacokinetics due to genetic variation in *CYP3A4* also observed (Maslub *et al.,* 2024). The trend of lower atorvastatin response due to *CYP3A4* (rs2242480) variant in Chinese population also observed (Peng *et al.,* 2018).

Decreasing function of *CYP3A4*1G* towards statins was also found in Skagen studies (Skagen, 2014). According to their further studies, they also observed gene-dose effect after using atorvastatin on the mean reduction of serum total cholesterol. On the other hand, they did not observe this effect in case of simvastatin treatment due to some potential substrate specific effects. Therefore, the current study is additional supports that the genotyping of allelic variants of *CYP3A4*4* and *CYP3A4*1G* suitable and affordable strategy for identifying those cardiac and dyslipidemic patients not responding to specific statin treatment due to these genetic polymorphisms. It has been identified in earlier studies that *CYP3A4*4* and *CYP3A4*1G* polymorphisms also involve in decrease activity of cortisol and reduce the fentanyl metabolism respectively (Elens *et al.,* 2013; Yuan *et al.,* 2015).

This is the first study in Pakistani cardiac and hyperlipidemic patients to explore the genetic polymorphism of *CYP3A4* (*CYP3A4*4* and *CYP3A4*1G*) and to examine the association of these allelic variations with variable statins response. Both *CYP3A4*4* and *CYP3A4*1G* have been reported in prior studies as significant loss-of-function alleles. The present study also supports the genotyping of allelic variants of *CYP3A4*4* and *CYP3A4*1G* suitable and affordable strategy for identifying those cardiac and dyslipidemic patients not responding to specific statin treatment due to these genetic polymorphisms. Reliability of allele specific gel based PCR for *CYP3A4* mutations also established for indigenous population

Limitations and future direction

Regardless of providing informative genotyping data of *CYP3A4*4* and *CYP3A4*1G* in indigenous population, current study has a number of shortcomings. First of all, that the selected population for this study was only patients admitted to hospital and no base line data available regarding lipid profile. Therefore, there is chance of some selection bias and lack of baseline measurements may influence the interpretation of allele-specific effects on lipid response. The other thing is the low patients count for current study, which is not enough to provide significant data regarding frequency distribution of *CYP3A4*4* and *CYP3A4*1G* allelic variants. Another limitation that could affect the scope of current study is that some other genes, such as *SLCO1B1*, *CYP3A5* and *ABCG2*, as well as clinical confounders (e.g., diabetes, BMI and concomitant medications) that could influence statin pharmacokinetics, were not studied or compared with the present results. So, to overcome these limitations there should be a large-scale study along with inclusion of control group required to support the findings of current study regarding *CYP3A4*4*

and *CYP3A4*1G* allele frequency distribution and also to obtain a more comprehensive understanding of the population distribution of these alleles and their clinical significance. Other allelic variants of *CYP3A4* gene like *CYP3A4*1B, CYP3A5*1, SLCO1B1* and *CYP3A4*22* should be explored as they also influence the statins effect.

CONCLUSION

The *CYP3A4* polymorphism is linked to variations in lipid profiles and may influence lipid response to statin therapy. However, these outcomes should be interpreted with caution, as the predictive value of these variants alone is inadequate. Once the findings of current study validated in larger cohorts, could be incorporated as part of a comprehensive pharmacogenetic panel alongside other genes with definitive confirmation, as suggested by AMP guidelines. Individualized statin therapy could be improved by providing a more accurate estimate of lipid response and dosage for better clinical outcomes.

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Authors' contributions
Amina Arif, Adil Jamal: Conceptualization, Methodology, Project administration, Supervision, Resources, Formal analysis, Writing-original draft, Writing-review and Final editing, validation; Hafiz Rafay Rawaal: Investigation, Data analysis, Data curation, Writing-review and Editing; Kashif- ur- Rehman: Methodology, in silico (software / computational analysis).

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Data availability statement
The data used to support the findings of this study are included in the article.

Ethical approval
This study was approved from Human Research Ethics Committee (HREC), Faculty of Science and Technology (FoST), University of Central Punjab, Lahore, Pakistan, Ref. #. FoST/DERC/2024/08 (Dated: 30-05-2024). This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

Conflict of interest
The authors declare that they have no known conflicts of interest related to this research article.

Supplementary data
https://www.pjps.pk/uploads/2026/05/SUP1779710624.pdf

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