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Precision Medicine Communications

DOI: doi.org/10.55627/pmc.003.002.0430

Research Article

Association of *GATM* Gene Polymorphisms with Statin-Induced Myopathy

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Abstract

Statins are one of the mainstay medicines for treating hyperlipidemia. The effect of statins can extend beyond their direct role in cholesterol transport to anti-inflammatory and plaque stabilization in coronary artery disease (CAD). Statins are known to reduce low-density lipoproteins (LDL) by up to 55%, which can inadvertently reduce the risk of cardiac events in patients. Strong clinical profile notwithstanding, statins are notorious for their muscle-related adverse effects. Some variations in genes such as glycine amidinotransferase (*GATM*) have been implicated in this side effect; however, the reports are not all congruent. In the current study, a prospective cohort design was used to establish both drug efficacy and the incidence of muscle-rated adverse reactions in patients receiving atorvastatin and rosuvastatin. The muscle adverse effects were recorded using the "Statin Associated Muscle Symptoms-Clinical Index" (SAMS-CI) tool. Drug plasma levels were recorded using LC-MS, and *GATM* rs9806699 was genotype using direct Sanger sequencing. A binary logistic model was used to determine the association between genotypes and the incidence of muscle symptoms, whereas stepwise linear regression was used to determine the association between plasma concentration, genetics, and muscle symptoms. Among 130 enrolled patients, 97(74.62%) received rosuvastatin 10 mg (once daily), and 33 (25.38%) received atorvastatin 20 mg (once daily). A total of 26 patients reported adverse drug reactions according to the SAMS-CI. The genotype frequency of *GATM* rs9806699-GG was 45.74%, whereas the heterozygous genotype (AG) was 34(36.17%), and the AA genotype was 18.09%. There was no significant difference found between the plasma concentrations of both rosuvastatin and atorvastatin among *GATM* rs9806699 genotypes. Despite the high incidence of muscular adverse effects, there was no significant association between *GATM* genotypes and SAMS.

Keywords: Atorvastatin; Rosuvastatin; Glycine amidinotransferase gene; Single nucleotide polymorphism; Statin-induced myopathy.

1. Introduction

Statins, 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase inhibitors, a prominent group of lipid-lowering drugs, are the most frequently prescribed drugs worldwide because they are the first-line therapy for managing hyperlipidemia and coronary heart disease (CHD) risk. Statins are generally effective in lowering low-density lipoprotein cholesterol (LDL-C) levels by up to 55% and cardiovascular events by up to 20% (Morofuji et al. 2022, Hussain et al. 2023).

Despite statins' well-established safety and efficacy, there is still a sizable inter-individual and inter-ethnic difference in how well they lower cholesterol. Nearly one-third of patients

do not achieve optimal outcomes, and 10% of patients experience statin-associated muscle symptoms (SAMS), which can range in severity from mild myalgia to life-threatening rhabdomyolysis. It has been reported that 10–15 % of statin users experience muscular problems, the most common of which is muscle pain. These rates have caused much debate and dispute because they are substantially higher than those observed in experimental studies (Mijajlović et al. 2017).

Serious muscular issues including rhabdomyolysis and statin-associated autoimmune myopathy affect < 0.1 % of statin users. Rhabdomyolysis can cause life-threatening kidney damage. Risk factors for an increase in statin-induced rhabdomyolysis include increasing age, certain medicines such as fibrates, and anti-thyroid (Peringat, Manappallil, and Karadan 2018). Statin usage reduces coenzyme Q10 (CoQ10 or ubiquinone) levels. Although proof of their effectiveness is insufficient, CoQ10 supplements are often prescribed to treat statin-associated myopathy (Strongin et al. 2017). Over 250,000 people received statin treatment between 1998 and 2001, and as per published reports, 0.44 out of every 10,000 of these statin users experienced rhabdomyolysis. The risk was almost ten times higher when cerivastatin was used or when traditional statins such as atorvastatin or rosuvastatin were combined with fenofibrate or gemfibrozil (Ho and Walker 2012).

Several studies have shown that lipophilic statins, such as atorvastatin, lovastatin, and simvastatin, are less dangerous than hydrophilic statins, such as fluvastatin, rosuvastatin, and pravastatin. Nevertheless, other investigations have not supported this hypothesis (Shattat and Journal 2015). Lovastatin increases the expression of the gene *atrogin-1*, which is thought to contribute to muscle fiber injury (Prathayini Paramanathan et al. 2021).

Glycine amidinotransferase (*GATM*) encodes the enzyme L-arginine: glycineamidinotransferase. This enzyme catalyzes the conversion of L-arginine into guanidinoacetate, which is a rate-limiting step in creatine synthesis (Wyss and Kaddurah-Daouk 2000). Mangravite et al. (Mangravite et al. 2013b) underlined the potential contribution of *GATM* genetic variants to SAMS. In a study, simvastatin was administered to lymphoblastoid cell lines, and the expression of different genes was measured. When *GATM* was exposed to simvastatin *in vitro*, its expression changed considerably. Subsequently, single nucleotide polymorphism (SNP) screening at the *GATM* locus revealed that rs9806699 was the most highly correlated differential deQTL. An association study of rs9806699 was performed in a 'two cohorts 'study to investigate the connection of this deQTL with statin-induced myopathy (SIM); the first compared 72 myopathy patients with 220 matched controls, and the second included 100 myopathy cases. Both investigations independently found a substantial link, and a meta-analysis revealed that the allele of rs9806699 was significantly related to a reduced risk of SIM (Mangravite et al. 2013b). However, such results could not replicate an association between *GATM* rs9806699 polymorphism and SIM in a large, multicenter case-control analysis of SIM in 715 hyperlipidemia patients (Luzum et al. 2015b). In Japan, too, the *GATM* polymorphism was not considerably related to SIM (Sai et al. 2016). Other studies regarding the effect of rs9806699 G > A on SIM yielded conflicting results (Liu et al. 2021, Carr et al. 2014, Floyd et al. 2014, Sai et al. 2016). Therefore, the role of *GATM* polymorphism in SIM remains a much-debated topic. The aim of the present study was to explore the relationship between *GATM* SNP rs9806699 G > A and SIM in a cohort receiving statins, in the province of Khyber Pakhtunkhwa, Pakistan. We hope that this investigation will help to identify high-risk populations for SIM and provide more individualized recommendations for statin users.

2. Materials and Methods

2.1. Study Design, Approval, and Duration

The current study was conducted using a longitudinal, prospective cohort design supported by experimental data. Patients with hyperlipidemia and those on statin therapy were enrolled after detailed medical checkups. The study protocol was approved and endorsed by the Khyber Medical University Advanced Studies and Research Board (AS&RB) (letter number DIR/KMU- AS&RB/DS/001014. The study follow-up duration was 24 weeks after the initiation of statin therapy. The study follow-up period was from March to October 2019. Patients aged above 30 with total cholesterol between 160 to mg/dl were included in the study, and patients currently using corticosteroids or immunosuppressant pregnant women were excluded from the study.

2.2. Study Protocol, Inclusion/Exclusion Criteria, Baseline Data

Patients were enrolled after a detailed selection procedure and were assessed at baseline after signing the informed consent. Blood samples were obtained in EDTA and whole blood and serum gel tubes. The first follow-up was conducted after six weeks of statin therapy initiation. The patients were called for a follow-up visit, and blood samples were collected in gel tubes for lipid profile and statin plasma level determination. During the first follow-up visit, patients were also assessed for muscular symptoms. Similarly, patients were followed up and called for a 2nd follow-up visit after six months of statin therapy initiation. Similar to the baseline and first follow-up visits, in the 2nd follow-up, patients were called for lipid profile determination and statin-associated muscle symptoms assessment.

2.3. Lipid Profile Measurement

The lipid profile was determined at baseline, after 6 weeks, and after 24 weeks of atorvastatin and rosuvastatin therapy. Blood samples were collected during the respective visits in gel tubes, and after coagulation, the tubes were centrifuged for serum separation. The serum was analyzed for lipid profile, including total cholesterol level, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels. A Cobas C111 biochemistry automatic analyzer (Gröschl and Doherty) was used for the determination of lipid parameters using appropriate kits and reagents as prescribed by the manufacturer.

2.4. Pharmacokinetics Analysis

The HPLC-MS method, developed and validated in one of our previous studies (Hussain et al. 2022), was used for the quantification of atorvastatin and rosuvastatin in blood samples. A mixture of methanol and water in a 70:30 ratio by volume was used as the mobile phase, and the pH was set at 3. The oven temperature was 25 °C and the sample injection volume was set at 1 μL. ESI-MS interface was operated in the positive ionization mode at block temp 400 °C, interface temp 350°C, DL temperature 250, CID gas Ar 270 kpa, Neb gas N2 flow at 2.0 L/min, drying gas N2 flow at 10 L/min, and heating gas Zero Air flow at 10.0 L/min.

After oral administration of a single dose of 40 mg atorvastatin, blood samples (5 mL) were collected in heparinized tubes at various time intervals (0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 12.00, and 24.00 h). Uncoagulated blood samples were centrifuged for 0.5 min at 4000 rpm to separate the plasma from cells and then stored at -20°C. Although the analytes were stable enough at ambient temperature, great care was taken to protect them from excessive heat and light during handling. Prior to analysis, plasma samples were thawed at ambient temperature, and pre-established extraction procedures were used for the extraction of drugs and their analysis using the LC-MS method. Pharmacokinetics (PK Summit) software was used to evaluate various parameters by performing non-compartmental pharmacokinetic analysis, and the data were

Table 1: Background characteristics and their association with statins groups.

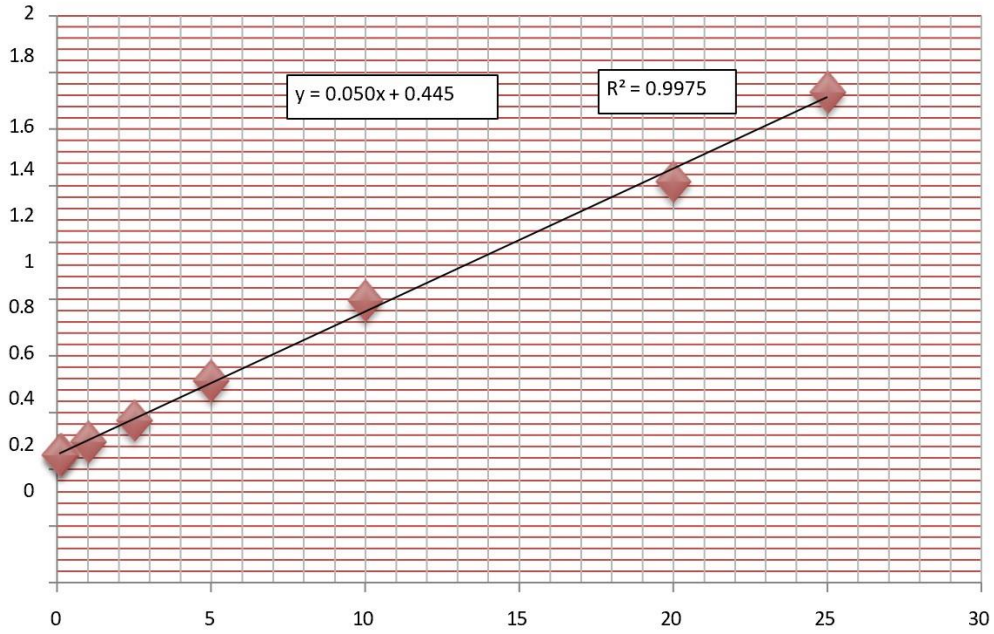
Characteristics		Total	Statin type		p-value
			Rosuvastatin N=97	Atorvastatin N=33	
Age Mean±SD		**53.58±9.88**	53.52±10.08	53.79±9.42	**0.892**
Age groups, n (%)	30-45years	**25(19.23)**	20(20.62)	5(15.15)	**0.417**
	46-60years	**78(60.00)**	55(56.7)	23(69.7)	
	≥60 years	**27(20.77)**	22(22.68)	5(15.15)	
Gender, n (%)	Female	**60(46.15)**	45(46.39)	15(45.45)	**1.000**
	Male	**70(53.85)**	52(53.61)	18(54.55)	
Weight (Kg), [(median (IQR)]		**78.00(10.25)**	78.45±7.31	78.61±9.07	**0.979¶**
Height (cm), [(median (IQR)]		**170.50(5.73)**	170.70±5.81	169.91±5.54	**0.313**
BMI, kg/m²		**27.08±2.64**	27.00±2.51	27.30±3.02	**0.579**
BMI cutoffs WHO, n (%)	Normal	**27(20.77)**	19(19.59)	8(24.24)	**0.359**
	Overweight	**80(61.54)**	63(64.95)	17(51.52)	
	Obese	**23(17.69)**	15(15.46)	8(24.24)	
BMI cutoffs Asia, n (%)	Normal	**5(3.85)**	3(3.09)	2(6.06)	**0.678**
	Overweight	**21(16.15)**	15(15.46)	6(18.18)	
	Obese	**104(80.00)**	79(81.44)	25(75.76)	
Currently Smoking, n (%)	No	**117(90.00)**	88(90.72)	29(87.88)	**0.738**
	Yes	**13(10.00)**	9(9.28)	4(12.12)	
Diabetes mellitus, n (%)	No	**106(81.54)**	80(82.47)	26(78.79)	**0.613**
	Yes	**24(18.46)**	17(17.53)	7(21.21)	
Cardiovascular disease, n (%)	**No**	**118(90.77)**	**91(93.81)**	**27(81.82)**	**0.074**

subjected to statistical analysis using MS Excel & Minitab software. The time to reach Cmax (Tmax) and maximum plasma concentrations (Cmax) were observed directly from the p (plasma) concentration versus time profiles, and the data were calculated for atorvastatin. The linear trapezoidal rule was followed for the determination of AUCs from initial t to final quantifiable conc. (AUC0–t)

2.5. Genetic Analyses

Blood samples were collected in EDTA tubes and stored at 2 to 8°C. Prior to DNA extraction, the tubes were left at room temperature for 30 min and gently inverted several times. A 2-ml microcentrifuge tube was used to combine 1 ml of RBC lysis buffer with 500 µl of blood using the reverse pipetting method. After allowing the mixture to incubate for 5 min, it was centrifuged for 3 min at 7000 rpm. The pellet was then re-suspended by gently overtaxing and reverse pipetting, and the supernatant was discarded. This process was repeated with 1 ml of the new RBC lysis buffer. To dissolve the pellet, 400 µl of nucleic extraction buffer was added, and reverse

Figure – 1: Calibration curve of Rosuvastatin



pipetting was performed. After the pellet was dissolved, 600 µl of refrigerated chloroform was added, and the mixture was gently vortexed and rested for 2 min. The distinct top and bottom layers were formed after centrifugation for 1 min at 14000 rpm. The top layer was then carefully removed using a pipette and placed in a new centrifuge tube. To condense the DNA, 1 ml of chilled 100% ethanol was added to the tube and stored at -20°C for 10 min. prior to use, absolute ethanol (99.7% purity) and 70% ethanol were also refrigerated at -20°C. DNA was stored at -20°C until ready for use in PCR or sequencing.

2.6. Sanger Sequencing

Targeted sequencing of *GATM* rs9806699 was performed on a Seq StudioTM genetic analyzer (Applied Biosystems, Waltham, Massachusetts, USA). Amplified PCR products with amplicon sizes of 385 bp were cycle-sequenced with a BDT v.3.1 (Applied Biosystems, Waltham, Massachusetts, USA) master mix according to the manufacturer's instructions for forward and reverse primers. The Big Dye X Terminator™ (Applied Biosystems, Waltham, Massachusetts, USA) kit was used to purify cycle-sequenced products. Samples were loaded into the Seq Studio TM genetic analyzer, and run parameters were set according to the kits used. The sequence data in the electropherogram was analyzed with Finch TV™ (v 1.4, Geospiza Inc., Seattle, WA, USA) genetic software, and SNP locations were visually checked for polymorphism. The *GATM* gene was studied for the known polymorphism rs9806699.

2.7. Assessment of Statin-Associated Muscle Symptoms

The assessment of SAMS involved the use of the SAMS Clinical Index (SAMS-CI), which adhered to the guidelines outlined by the National Lipid Association (NLA) Statin Muscle Safety Task Force of the United States. The SAMS-CI provided an objective and rigorous evaluation of SAMS based on four criteria that pertain to the location and pattern of pain, onset of symptoms, symptom resolution upon cessation of statin therapy, and recurrence of symptoms upon re-initiation of statin therapy(Rosenson et al. 2017). A structured Proforma and patient information form were used to systematically collect data on muscular symptoms.

2.8. Statistical Analysis

Data were statistically analyzed using SPSS (version 21®) and GraphPad Prism. The mean, standard deviation (SD), maximum, minimum,

Table 2: Calibration curve for Analysis of Rosuvastatin

Data File	Conc. (ng/ml)	Drug area	I.S area	Area ratio	Calc conc.	Accuracy
	25	25752	14887	1.7298314	25.697	**102.79**
5	**20**	23702	16739	1.4159747	19.419	**97.10**
7	**10**	16449	16506	0.9965467	11.031	**110.31**
8	**5**	11378	15952	0.7132648	5.365	**107.31**
9	**2.5**	10352	18045	0.5736769	2.574	**102.94**
10	**1**	8824	17752	0.4970708	1.041	**104.14**
13	**0.1**	8621	19171	0.4496896	0.094	**93.79**
14	**0.05**	**8188**	**18298**	**0.4474806**	**0.050**	**99.22**

median, and interquartile ranges were used to present continuous variables. Categorical variables are expressed as frequency and percentage. The difference between expected and observed frequencies was calculated using the chi-square test and analysis of variance (ANOVA) was used to determine differences between numerical variables for variables with more than two.

3. Results

A total of 130 hyperlipidemic patients were included in the final analysis; their mean (SD) age was 53.58 ± 9.88 years, ranging from 30 to 76 years. The majority was aged between 46 and 60, and there was a male preponderance. Anthropometric data revealed a median weight of 78.00 (Interquartile range (IQR) 10.25) kg. Similarly, the mean body mass index (BMI) was calculated to be 27.08 ± 2.64 kg/m2. The BMI cutoffs were determined based on the World Health Organization's recommended cut-points for the Asian population. According to WHO cutoffs, the majority of patients were overweight (61.54%, n = 80), while using Asian cutoffs as a reference, 80% of participants were classified as obese. Diabetes mellitus (DM) was the most prevalent co-existing illness, and the proportion of smokers was 10%.

The majority of the patients were taking rosuvastatin (10mg, once daily) while 25.33% were on atorvastatin. Patients 'background characteristics were compared between the statin groups. Both groups were comparable in terms of background characteristics and overall, no significant differences in background characteristics were observed between rosuvastatin and atorvastatin. Additional details are provided in Table 1.

Determination of plasma level of atorvastatin and rosuvastatin was carried out using LC coupled with MS. Validated and developed method for simultaneous analysis was carried out as per standard protocols (USP and ICH guidelines table 2). A representative chromatograph of statin from patients' plasma at a steady state following morning dose is shown in Figure 2.

Patients treated with rosuvastatin (10mg) had a mean plasma level of 7.06±2.05 ng/ml, while the patients treated with atorvastatin (20mg) had a mean plasma level of 7.39±2.35 ng/ml. Mean plasma differences were calculated using an independent t-test and one-way analysis of variance (One-way ANOVA). The mean plasma level did not differ significantly across the background characteristics.

As shown in Table 3, the distribution of different genotypes of the *GATM* gene did not exhibit a

Table 3: Allele and genotype distribution of *GATM* (rs9806699) gene polymorphisms in hyperlipidemic patients In Khyber Pakhtunkhwa.

Study population with corresponding numbers of antihyperlipidemic drugs	Genotype						Allele		
	AA, n (%)	AG, n (%)	GG, n (%)	χ^2	Df	p-value ¶	G, %	A, %	p-value †
Total study sample (N=94)	17(18.09)	34(36.17)	43(45.74)	4.412	1	0.035	0.64	0.36	**0.827**
Patients treated with rosuvastatin (N=67)	13(19.40)	24(35.82)	30(44.78)	3.677	1	0.055	0.63	0.37	
Patients treated with atorvastatin (N=27)	4(14.81)	10(37.04)	13(48.15)	0.075	1	0.386	0.67	0.33	

¶ Calculated online using Hardy-Weinberg Equilibrium (HWE) Calculator
†Chi square statistics is computed for p-value

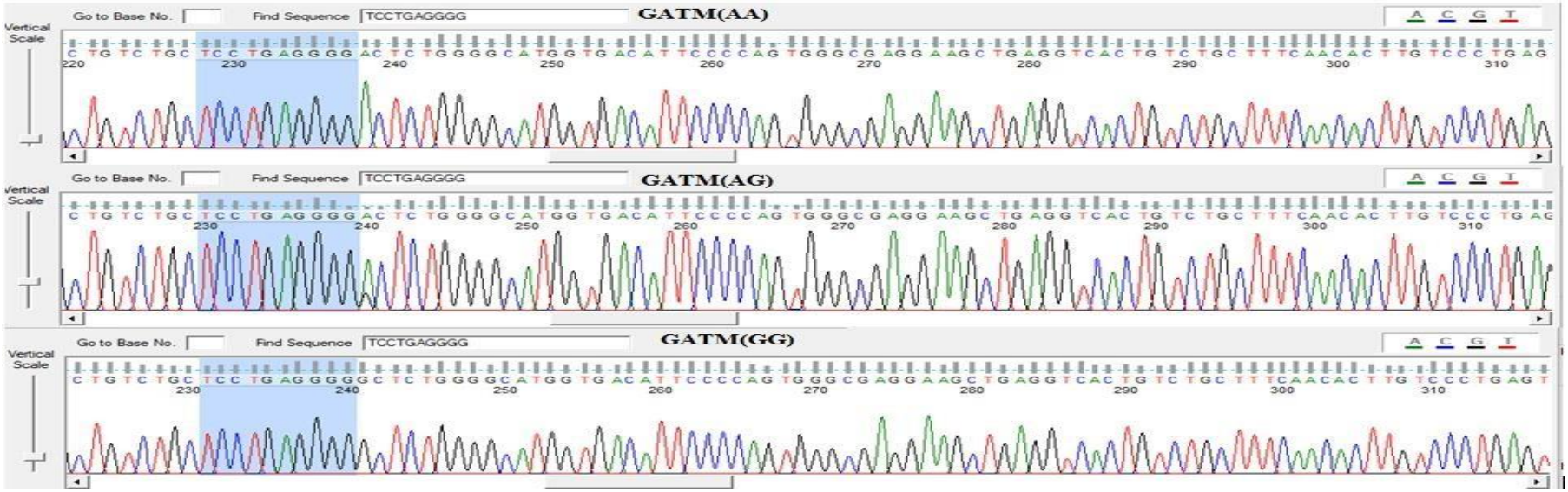


Figure 2: Electropherogram of targeted sequencing of *GATM* rs9806699 (G>A). A: Reference homozygous AA, B: heterozygous AG, C: minor allele homozygous GG

significant variation (P>0.05), the observed genotype distribution is in confirmation of Hardy-Weinberg proportions. The allelic frequency of the (A) allele and (G) allele in the *GATM* gene were low and high respectively. The frequency of wild-type homozygous genotype (AA) in the study population using rosuvastatin and atorvastatin was 19% and 14% respectively, while the frequency of heterozygous mutant (AG) was 35% and 37%, and homozygous minor allele frequency was 44% and 48% in rosuvastatin and atorvastatin treated groups. There was no significant difference observed in the distribution of alleles among the different groups (p= 0.827).

Using one-way ANOVA, the plasma concentration of Rosuvastatin versus gene polymorphisms was determined as shown in table 4. The heterozygous genotype (AG) had a significantly lower mean plasma concentration as compared to the wild homozygous allele (AA) and minor homozygous allele (GG) and the difference was statistically insignificant (p=0.427). Similar results were observed for atorvastatin.

As shown in Table 5, changes in lipid parameters versus *GATM* rs9806699 gene polymorphisms in patients using rosuvastatin were determined. Among the lipid parameters, there was a statistically significant difference for TC (p<0.001) and LDL (p<0.001) in patients with AG genotype as determined by one-way ANOVA. Similarly, significant differences were observed in patients with AA genotypes and other groups for TC and LDL (p>0.001). Likewise, statistically significant mean differences between GG and other groups were observed for TC and LDL.

Post-hoc Tukey's test for lipid parameters at baseline and follow-ups revealed that for patients with AG genotype, the TC and LDL were significantly lower for the first follow-up in rosuvastatin users compared to baseline (p<0.001) and the second follow-up compared to the baseline (p<0.001). LDL in patients with the AA genotype showed a significant difference between baseline vs first follow-up as well as second follow-up. There was a statistically significant difference between baseline vs first follow-up and baseline vs second follow-up in TC and LDL levels for patients with GG genotype (p <0.001). TG level differed only in baseline vs second follow-up in patients with GG genotype.

In patients treated with atorvastatin, there was a statistically significant difference for TC (p=0.036) and LDL (p=0.031) in AG and GG carriers. No significant differences were observed in AA carriers for TC HDL, TG, and LDL (p>0.05). Multiple comparisons using post-hoc Tukey's test for lipids parameters at baseline and follow-ups in patients treated with Atorvastatin are given in Table 5. A Tukey test revealed that in AG carriers, TC was statistically significantly lower in the second follow-up vs baseline (p=0.030). Furthermore, similar results were noted for LDL in patients with the AG genotype which showed a significant difference between the second follow-up vs baseline (p =0.026). In GG carriers, TC and LDL showed significant differences in baseline vs first follow-up as well as second follow-up (p<0.001).

SAMS were observed in 22.40% of the patients, while at the second follow up the SAMS increased to 35.5%. Those who were using rosuvastatin had a higher proportion of SAMA compared to atorvastatin at the first follow-up as shown in Figure 3, however, no significant difference was noted at the second follow-up.

The presence and absence of SAMS were cross-tabulated with background characteristics and the type of statins used by patients. Comparable mean age was observed in both groups. Similarly, no association was observed for gender. Weight was comparable between the groups as well as BMI. Similar results were observed for co-morbid conditions except diabetes mellitus. The proportion of patients who reported having a higher number of ADRs

Table 4: Plasma concentration/levels of statin versus gene polymorphisms.

Gene		Plasma level of statin						p-value
		Mean	SD	Median	Range	Minimum	Maximum	
GATM rs9806699, (Rosuvastatin)	AG	6.74	1.47	6.21	4.46	4.76	9.22	**0.427**
	AA	6.93	1.49	7.20	4.59	4.01	8.60	
	GG	7.47	2.59	7.73	12.93	3.53	16.46	
GATM rs9806699, (Atorvastatin)	AG	7.60	1.69	8.29	4.14	5.17	9.31	**0.855**
	AA	6.87	1.08	6.74	2.58	5.70	8.28	
	GG	**7.64**	**3.16**	**7.49**	**10.41**	**1.32**	**11.73**	

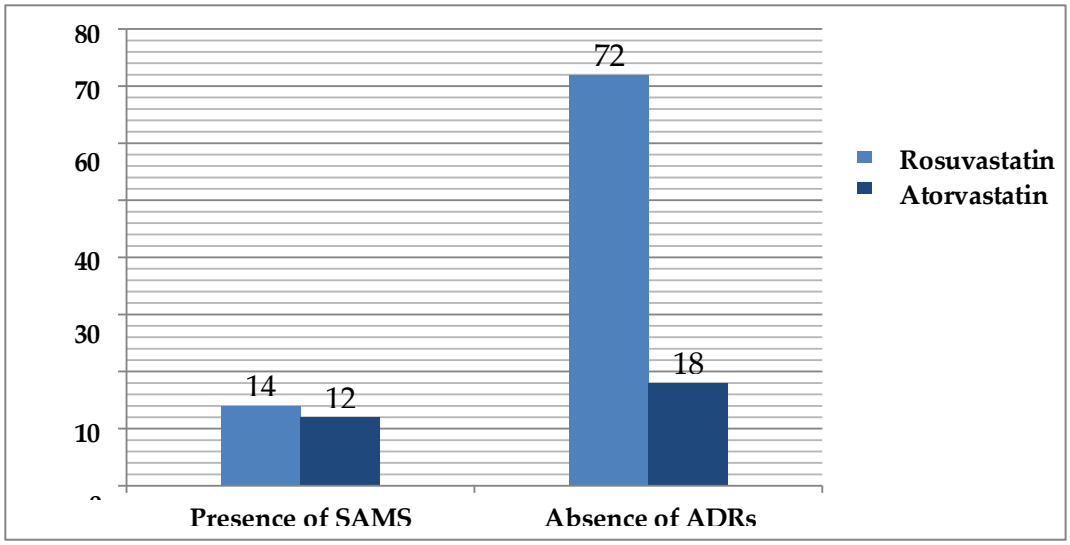


Figure 3: presence or absence of ADRs based on type of treatment.

was using rosuvastatin compared to atorvastatin and, a statistically significant association was noted between them (p=0.011).

The mean plasma level of the drug was compared between the patients with and without ADRs using t-statistics for the total population. A significant mean difference was observed between them (p>0.05). The distributions of plasma levels in patients with and without ADRs are given in Figure 4. The seriousness of SAMS was equally common in both groups of patients. Plasma levels were different between them, and a significant mean difference was noted (p<0.001). Furthermore, patients with AG (nearly 82%) had no ADRs. None of the gene polymorphisms showed statistically significant associations with SAMS. Moreover, cross-tabulation of responses to statins and SAMS showed no significant association.

4. Discussion

In the present study, we analyzed the correlation between genetic polymorphism and the risk of SAMS within the satin-treated sample. The protective effect of *GATM* rs9806699 G > A was not replicated in our study population. *GATM* (rs9806699) had a higher number of participants with genotype (GG) 43 (45.74%). The heterozygous genotype (AG) frequency was 36.17% (n=34). Mutant homozygosity was observed in 17 (18.09%). Similarly, in our study, the minor allele frequency observed for the rs9806699 was 0.36, nearly in line with findings

Table 5: Changes in lipids parameters versus *GATM* rs9806699 gene polymorphisms in patients treated with statin

Lipids	*GATM* rs9806699											
	AG				**AA**				GG			
Rosuvastatin												
	Baseline	**6 weeks**	**6 months**	**p**	**Baseline**	**6** weeks	**6 months**	**p**	**Baseline**	**6 weeks**	**6 months**	p
TC	213.37±27.95	179.20±29.07	168.66±23.15	0.000	214.23±26.55	179.84±28.51	161.23±15.74	0.000	216.83±36.24	182.93±37.21	168.40±26.18	**0.000**
HDL	36.37±6.80	38.33±8.18	37.45±4.23	0.592	39.46±6.35	41.61±6.33	40.15±4.09	0.620	38.26±7.68	40.20±7.71	39.33±4.85	**0.554**
TGs	230.08±63.43	192.45±63.07	212.00±69.82	0.146	215.92±82.54	191.69±59.65	200.76±62.17	0.666	240.530±66.08	207.43±70.69	196.76±54.84	**0.026**
LDL	130.98±22.14	102.38±27.32	88.80±23.30	0.000	131.58±21.58	99.89±21.13	80.92±15.08	0.000	130.46±29.54	101.24±29.51	89.71±20.78	**0.000**
Atorvastatin												
TC	213.50±31.60	188.90±39.28	174.70±23.12	0.036	211.25±33.05	185.50±37.96	173.75±22.63	0.278	213.92±26.47	169.38±19.28	161.00±15.71	**0.000**
HDL	35.10±4.43	37.20±4.18	37.40±3.33	0.378	32.50±2.88	36.00±5.03	36.25±2.87	0.330	36.38±4.89	39.30±5.93	38.84±4.27	**0.301**
TGs	230.10±46.74	205.60±82.47	193.50±63.13	0.458	273.50±68.61	254.00±67.23	216.75±84.94	0.566	262.46±63.54	230.69±55.99	206.92±67.49	**0.090**
LDL	**132.38±28.37**	**110.58±32.29**	**98.60±19.14**	**0.031**	**124.05±16.50**	**98.70±24.97**	**94.15±16.23**	**0.122**	**125.04±16.73**	**83.93±12.54**	**80.76±6.43**	0.000

observed in South Asian populations (0.40) for the G allele (https://genome.ucsc.edu/). In the European population, the allelic frequency was observed to be 0.723, whereas the alternate frequency was found to be 0.276. The African population had a distribution of 0.811 for the (A) allele and 0.188 for the G allele (https://genome.ucsc.edu/).

SAMS are the most frequently reported adverse effects of statin therapy(Rosenson et al. 2014). Of the total, ADRs were observed in 22.40% of the patients. Those who were using rosuvastatin had a higher proportion of ADRs compared to atorvastatin (p=1.000) at the first follow-up; however, no significant difference was noted at the second follow-up. The presence and absence of ADRs were cross-tabulated with background characteristics. Comparable mean age was observed in both groups. Similarly, no association was observed between gender. Weight was comparable between the groups as was BMI. Similar results were observed for comorbid conditions except diabetes mellitus. Karalis et al. (2016) reported SAMS in 31% of females and 26% of males, which is in discordance with our observations (Karalis et al. 2016). (HPCG 2002) have also reported a higher incidence of SAMS in women than in men, while another latest study reported a higher incidence of symptoms in male patients (31.4%) than in females (22.6%), which is similar to our observations (Abed et al. 2022).

Although not statistically significant, there was a trend of increasing the odds of myopathy with increasing age, as 14.3%, 26.3%, and 27.6% of patients suffered from SAMS in the 30–45, 46–60, and 61–76 years age groups, respectively. This phenomenon has also been reported by other researchers (HPCG 2002). In our study cohort, n=31(24.6%) of the participants based on the 6th-week follow-up visit assessment reported muscle pain due to this new treatment. Based on the SAMS-CI score, 8.8% of the total study participants (n=11) initially reported to have statin-induced pain were ―Unlikely‖ to have SAMS, while 8.00% (n=10) ―Possibly‖ had the pain due to statins. Patients with a SAMS-CI score of 9-11 were 5% of the total patients initially considered to have statin-induced muscle symptoms. Cohen et al. (2012) reported SAMS in 29% of the statin users, 33% of these had stopped statins without consulting their physician, and 62% of the former users had stopped statins because of muscle pain (Cohen et al. 2012). Bruckert et al. in the Prediction of Muscular Risk in Observational conditions (PRIMO)‖ reported that 10% of the statins 'users experienced muscle symptoms. (Bruckert et al. 2005).

SAMS are reported to have a strong association with gene polymorphisms (Stroes et al. 2015). A genome-wide association study examined *GATM* G>A (rs9806699) as a potential genetic marker for the lower risk of SIM. The *GATM* rs9806699 allele has a protective effect on SIM by causing a higher decline in *GATM* RNA expression (Bai et al. 2018, Mangravite et al. 2013a). As per the hypothesis of Mangravite et al. the cause of protective function is the slowing down of cellular processes that are needed for the development of SIM. Additionally, the cause of slowing the cellular processes is the dramatic decrease in the capacity of muscle cells in the form of very low levels of phosphocreatine related to the variant allele of *GATM* (Luzum et al. 2015a). In our study, patients with the A allele for *GATM* rs9806699 had a lower probability of developing SIMS. According to Xue Bai et al.., *GATM* rs9806699 was tangentially related to SIM occurrences (Bai et al. 2019). The effects of *GATM* rs9806699 on SIM were unrelated to the high plasma exposure of rosuvastatin and its metabolites and were therefore suitable indicators for predicting rosuvastatin-induced myopathy.

A comparable result was observed for *ABCB1* rs1128503 SNP, where the proportion of heterozygous allele AG was 36(85.71%) and the minor homozygous allele was GG 2(25.00%). The proportion of wild homozygous was nearly

12(37.50%) in those with SAMS. Similar findings were observed for *ABCG2* rs22311420, where the proportion of patients with SNP GT and ADRs was 16.67%.

5. Conclusions

In conclusion, our cohort study has indicated that *GATM* polymorphism is not associated with the risk of SIM. Variations including rs9806699 G>A, may not be protective factors of SIM. The association of rs9806699 G>A with severe SIM was non-significant, indicating that it may only exert a protective effect on mild SIM cases.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

This project did not receive any external funding.

Study Approval

The study protocol was approved and endorsed by the Khyber Medical University Advanced Studies and Research Board (AS&RB) (letter number DIR/KMU- AS&RB/DS/001014.

Consent Forms

All patients signed consent forms which are available with the authors.

Authors Contribution

SAT conceptualized the study, AH, MZ, and MAR helped in the literature review and experimental work, JA and MFR helped in analysis, and interpretation and helped write the first draft, SAT supervised the whole project and wrote the final manuscript.

Acknowledgments

We thank the Institute of Pharmaceutical Sciences, Khyber Medical University, Peshawar for their support in performing this investigation.

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