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Implication of *MATE2*, *OCT2* and *ATM* Genes Polymorphism and Their Association with Metformin Efficacy and Glycemic Control in Type 2 Diabetes Mellitus Patients

(Implikasi Polimorfisme Gen MATE2, OCT2 dan ATM serta Perkaitannya dengan Keberkesanan Metformin dan Kawalan Glukosa dalam Kalangan Pesakit Diabetes Mellitus Jenis 2)

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ABSTRACT

In type 2 diabetes mellitus (T2DM) patients, metformin, the drug of choice, exhibits variable therapeutic response attributed to gene polymorphism. The current study aimed to investigate the association of MATE2 rs12943590, OCT2 rs7757336, and ATM rs11212617, which are hotspot single-nucleotide polymorphisms (SNPs), with metformin efficacy and glycemic control in T2DM patients. A total of 417 study subjects were enrolled, consisting of 217 newly diagnosed and drug-naive T2DM patients, while 200 individuals were healthy controls. Patients were further divided into two subgroups: metformin responsive and metformin-non-responsive individuals. Study patients were kept on exclusive metformin monotherapy for three successive months. Patients' basic parameters like age, fasting glucose, HbA1c, LDL, HDL, Cholesterol, and BMI were recorded. Real-time PCR involving melt curve analysis and subsequent agarose gel electrophoresis with Sanger sequencing was employed for genotype analysis. *MATE2* (*SLC47A2*) rs12943590 GA genotype (OR 0.44, CI 95% 0.23-0.86, $p = 0.01$), *OCT2* (*SLC22A2*) rs7757336 GG genotype (OR 5.82, 95% CI 1.40-24.24, $p = 0.01$), and G allele (OR 2.21, CI 95% 1.18-4.14, $p = 0.01$) were significantly associated with metformin response and glucose-lowering effect. No significant association ($p > 0.05$) was observed for *ATM* rs11212617.

Keywords: Association studies; diabetes mellitus; gene polymorphism; glycemic control; metformin

ABSTRAK

Metformin sebagai ubat pilihan untuk pesakit diabetes mellitus jenis 2 (T2DM) menunjukkan respons terapeutik yang berbeza-beza yang dikaitkan dengan polimorfisme gen. Penyelidikan ini bertujuan untuk mengkaji hubungan antara *MATE2* rs12943590, *OCT2* rs7757336 dan *ATM* rs11212617 yang merupakan polimorfisme nukleotida tunggal (SNP) titik panas dan hubungan mereka dengan keberkesanan metformin dan kawalan glisemik pada pesakit T2DM. Sebanyak 417 individu subjek kajian telah disertakan, terdiri daripada 217 pesakit T2DM yang baru didiagnosis dan tidak pernah menjalani rawatan, manakala 200 individu merupakan kumpulan kawalan sihat. Pesakit dibahagikan kepada dua kumpulan sub, iaitu individu yang responsif terhadap metformin dan individu yang tidak responsif terhadap metformin. Pesakit kajian diberikan monoterapi metformin eksklusif selama tiga bulan berturut-turut. Parameter asas pesakit seperti umur, glukosa puasa, HbA1c, LDL, HDL, kolesterol dan BMI dicatat. PCR masa nyata yang melibatkan analisis lengkung lebur dan elektroforesis gel agarosa seterusnya dengan penjujukan Sanger digunakan untuk analisis genotip. Genotip *MATE2* (*SLC47A2*) rs12943590 GA (OR 0.44, CI 95% 0.23-0.86, p = 0.01), genotip *OCT2* (*SLC22A2*) rs7757336 GG (OR 5.82, CI 95% 1.40-24.24, p = 0.01) dan alel G (OR 2.21, CI 95% 1.18-4.14, p = 0.01) didapati secara signifikan berkaitan dengan respons metformin dan kesan penurunan glukosa. Tiada hubungan signifikan ($p > 0.05$) yang diperhatikan untuk *ATM* rs11212617.

Kata kunci: Diabetes mellitus; kajian hubungan; kawalan glisemik; metformin; polimorfisme gen

INTRODUCTION

Diabetes mellitus (DM), particularly type 2, is a complex disease with a surprising and alarming number of patients, recently over half a billion around the world (Guzman-Vilca & Carrillo-Larco, 2025; Saeedi et al. 2019). Clinical risks associated with T2DM include, but are not limited to, lower limb amputation, kidney failure, and developing blindness. Patients with T2DM face a relatively higher risk for cardiovascular diseases like stroke and heart attack (Alwan 2011; Chen et al. 2024). The trend in mortality in T2DM individuals is double that of their age and gender matched non-diabetic healthy peers (Van Dooren et al. 2013). It is predicted that by 2030, diabetes will be the seventh leading cause of death globally (Strom & Egede 2012).

The first line of drug prescribed in T2DM, considered a gold standard and over the counter with the highest safety profile yet less expensive, is metformin only (Lv & Guo 2020). Metformin belongs to the class of drugs called biguanides and is a drug of choice as per the American Diabetic Association (ADA) and the European Association for the Study of Diabetes (EASD) (Amin 2018; Baker et al. 2021; Davies et al. 2018). Metformin, being an antihyperglycemic drug, essentially causes a lowering of blood glucose levels to control elevated blood glucose levels in diabetic patients (Bailey 2024). The drug is approved by the FDA (Food and Drug Administration), being highly efficacious in T2DM (Foretz, Guigas & Viollet 2019; Kuhlmann et al. 2021). Since metformin is excreted without hepatic metabolism in unchanged form, therefore, its absolute mechanism is still elusive, although various mechanisms have been proposed. The general mechanism proposed for metformin action is that it reduces hepatic release of glucose, decreases the absorption of glucose in the intestines, sensitizes insulin, and promotes the efficient utilization of glucose in the peripheral tissues (Kuhlmann et al. 2021).

Metformin is being used by about 120 million T2DM patients all over the world. Beside the fact that metformin has a great efficacy and safety profile in the general population, about one out of three patients does not show adequate response to metformin when subjected to its monotherapy. Recent studies on pharmacogenomics showed that gene polymorphisms in the genes of drug metabolizing enzymes or drug transporters are responsible for the metformin response in various individuals based on their genetic profiles (Damanhouri et al. 2023). The concept of gene polymorphism is of special importance when there is a large sample size and diverse ethnicities are involved, isolated miles away. Various ethnicities have different gene pools established over the years. As described, metformin is not metabolized through routine hepatic metabolism, therefore, its transporters and the phenomenon of their gene polymorphism is of great importance to researchers these days (Al-Eitan et al. 2019).

The transport of metformin inside the body across the barriers is controlled by different genes that code for the respective transport proteins. These genes include multidrug and toxin extruders (*MATEs*), organic cation transporters (*OCTs*), and plasma membrane monoamine transporters (*PMAT*). The transporter PMAT is involved in the absorption of metformin, encoded by the gene *SLC29A4,* and *OCT3,* encoded by the *SLC22A3* gene present on the surface of enterocytes. Likewise, OCT1 occurs at the basolateral side of the enterocytes, encoded by the gene *SLC22A1* and transports metformin to the general body (Liang & Giacomini 2017). In the liver, metformin is transported by OCT1 and is excreted by renal mechanisms through MATE1 and MATE2 encoded by the *SLC47A2* gene (Jensen et al. 2016). OCT2, which is organic cation transporter 2, located at the basolateral side of the renal tubule, carries out the transportation or excretion of metformin. MATE2 is encoded by *SLC47A2* and is expressed in the distal and proximal renal tubules (Ito et al. 2012; Liang & Giacomini 2017). It is localized at chromosome 17p11.2. MATE2 is involved in the transport of metformin from tubular cells into the urine. Various SNPs of *MATE2* have been associated with metformin pharmacokinetics with strong effects (Choi et al. 2011; Kajiwara et al. 2009). *ATM* is located on chromosome 11q22-23, and it has a total of 66 exons. It encodes a 13 kb protein (Shokri et al. 2016). *ATM* gene is considered to play a role in DNA repair, AMP (adenosine monophosphate) activation of protein kinase, which is linked to affect glycemic control in T2DM (GoDARTS et al. 2011). A mutation in the ataxia telangiectasia mutated (*ATM*) gene is known to have manifested insulin resistance in T2DM (Altall et al. 2019; Vilvanathan et al. 2014). Moreover, it is estimated that about 90% of disease risk associated loci identified by genome wide association study (GWAS) are located in non-coding regions of the genome (Dong et al. 2023).

Previously, gene polymorphism has been shown to be associated with altered metformin pharmacokinetics. A GWAS has shown that the *ATM* gene is sufficiently associated with metformin pharmacokinetics (Damarov et al. 2024). Although various studies have associated gene polymorphism with metformin pharmacokinetics but there exists inconsistency in the results (Mohammadi Jouabadi et al. 2024; Saiz-Rodríguez et al. 2023). This inconsistency is attributed to different ethnicities and large sample populations (Dujic et al. 2017; Naesa & Joujeh 2024; Saiz-Rodríguez et al. 2023). In the current study, we investigated the association of *MATE2* rs12943590, *OCT2* rs7757336, and *ATM* rs11212617 gene polymorphism with metformin efficacy and glycemic control in T2DM patients. This study is expected to add to the efforts towards tailored personalized precision medicine based on an individual's pharmacogenomics profile.

MATERIALS AND METHODS

STUDY DESIGN AND SAMPLING

This study consisted of 417 total study subjects who were initially enrolled. Among the enrolled subject individuals, 200 (108 males and 92 females) were healthy controls, while 217 (103 males and 114 females) were newly diagnosed T2DM drug-naive patients according to the criteria of WHO 1999 (Altall et al. 2019; Lemeshow et al. 1990). All study subjects were unrelated and aged between 20 and 60 years. Study subjects were taken from the Pakistani province Khyber Pakhtunkhwa with Pashtun ethnicity, while patients with mixed ethnicity were excluded. Study subjects with conditions like myocardial infarction, liver and kidney dysfunction, cancer, stroke, and pregnancy were also excluded from the study. Patients were recruited from HMC Hospital Peshawar, a large tertiary care hospital.

All the subjects were categorized into three subgroups: controls, metformin-responsive individuals, and metformin-non-responsive individuals. Patients with T2DM were enrolled in a three-month metformin monotherapy and a follow-up checkup after three months. Basic information like gender, age, BMI (Body Mass Index), and family history were recorded. A prior written and duly signed consent was taken from all study subject on a predesigned form. The study was approved by the concerned ethical review board of the University of Peshawar, Pakistan (316/EC/F.LIFE/UOP-2020).

DETERMINATION OF CLINICAL PARAMETERS

For the determination of various biochemical parameters and onward DNA extraction peripheral whole blood of subject individuals was taken. The blood was stored in EDTA tubes at -20 °C for onward examination. All the related biochemical parameters like BMI, Fasting Blood glucose (FBG), glycosylated hemoglobin (HbA1c), and lipid profile were determined initially at the time of diagnosis and after three months of metformin monotherapy to cross-check the variations.

Recruited T2DM patients who responded to metformin and whose decrease in HbA1c was greater than 0.5 percent compared to the baseline set were grouped as metformin responders. Those patients whose HbA1c increased with a value of more than 0.5 percent from their baseline were called as metformin non-responders (Mahrooz et al. 2015; Umamaheswaran et al. 2015). Clinical analyzer Cobas® 6000 (Roche Diagnostics, Germany) was used for the determination of HbA1c and various lipid profile indicators like LDL (Low Density Lipoproteins), TC (Total Cholesterol), HDL (High Density Lipoproteins) and TG (Triglycerides).

DNA EXTRACTION, GENOTYPING, AND SANGER SEQUENCING

Before genotyping and sequencing, genomic DNA of all study subject participants was extracted with the phenol-chloroform method and quantified using a nanodrop NP80 ® (Implen, Germany). For genotyping allele-specific confronting pairs of primers utilizing Amplification-Refractory Mutation System (ARMS) PCR was used, while outer products were used for Sanger sequencing employing primer-1 (Collins & Ke 2012) (Table 1). Real-time PCR (RT-PCR) BIO-RAD CFX connect™ (Optics module, Singapore) was used for genotyping analysis. In RT-PCR, BIO-RAD CFX Maestro software 2.3 (version 5.03.022.1030) was used for analysis of the amplification and subsequent melt curve analysis for any non-specific amplification or allele discrimination. For confirmation purposes, the allele amplicons were analyzed on a 1.5% agarose gel electrophoresis and finally visualized using a BIO-RAD imaging system (chemiDoc™ Singapore) (Figure 1) (supplementary materials Figure S1-S5). PCR outer products Sanger sequencing was performed with random samples utilizing Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific, USA). For sequencing data analysis, Bioedit sequence alignment editor (BioEdit version 7.7.1) was used.

STATISTICAL DATA ANALYSIS

MedCalc software version 19.2 (MedCalc, Ostend, Belgium) and SPSS 18.0 (Chicago, Illinois, USA) were used for statistical data analysis. Logistic regression analysis was employed to find out the association of respective alleles and Single Nucleotide Polymorphism (SNPs) in the context of metformin efficacy in T2DM and control individuals. Those correlations with a $p \leq 0.05$ with a 95% confidence interval were considered as significant. Metformin responders and non-responders, alleles and genotypes were compared through odd ratios (OR). All other biochemical parameters values were expressed as mean ± SD.

RESULTS AND DISCUSSION

DEMOGRAPHICS AND CHARACTERISTIC FEATURES OF THE SUBJECTS

At the sampling stage, a total of 250 T2DM study subjects were recruited in the study. All patients were diagnosed for the first time with T2DM, thus, drug naive for T2DM and onward on metformin monotherapy only. As a control group, 200 age-matched healthy individuals were also recruited for the study. As per standard physicians' practice, patients were also advised on lifestyle interventions and food habits by the respective physicians. Individuals from either gender were present in a suitable number as mentioned in Figure 2(a) and 2(b).

EVALUATION OF CLINICAL PARAMETERS BETWEEN RESPONDERS AND NON-RESPONDERS

Based on metformin response in subject individuals, the total 217 patients were grouped as metformin responders and metformin non-responders. Responders were 157,

TABLE 1. PCR primers used for genotyping and Sanger sequencing

SNP	Primer name	Primer sequence (5′→3′)	Size (bp)
	FIA	GAGTAAGGGGCAGGAGGATGA	163
MATE2	RIG	CCACAAGTTGCCATGGTATCC	220
rs12943590	FO	CTCAGTCCCAAAGCCTCTGG	362
	RO	CCTCTGGGAGACCAGACACAA	
	FIG	TTCCCCTAACACTTTCCTCTGG	147
OCT2	RIT	CCCAGATCCACCAGGGAA	222
rs7757336	FO	ACAGCCTGTAATATCCAACAATGG	330
	RO	TTAACGTGATGTGGAGAGGGAA	
	FO	CTTTGCTTTCATTTGGGTT	343
ATM	RO	AGGTTCGTCTTTGTTCTTTTC	
rs11212617	FIA	CAAAGGGCAGATCAGAAAA	127
	RIC	GATTTTTTATCCGCTCTGATAG	256

F: Forward, O: Outer, R: Reverse, I: Inner

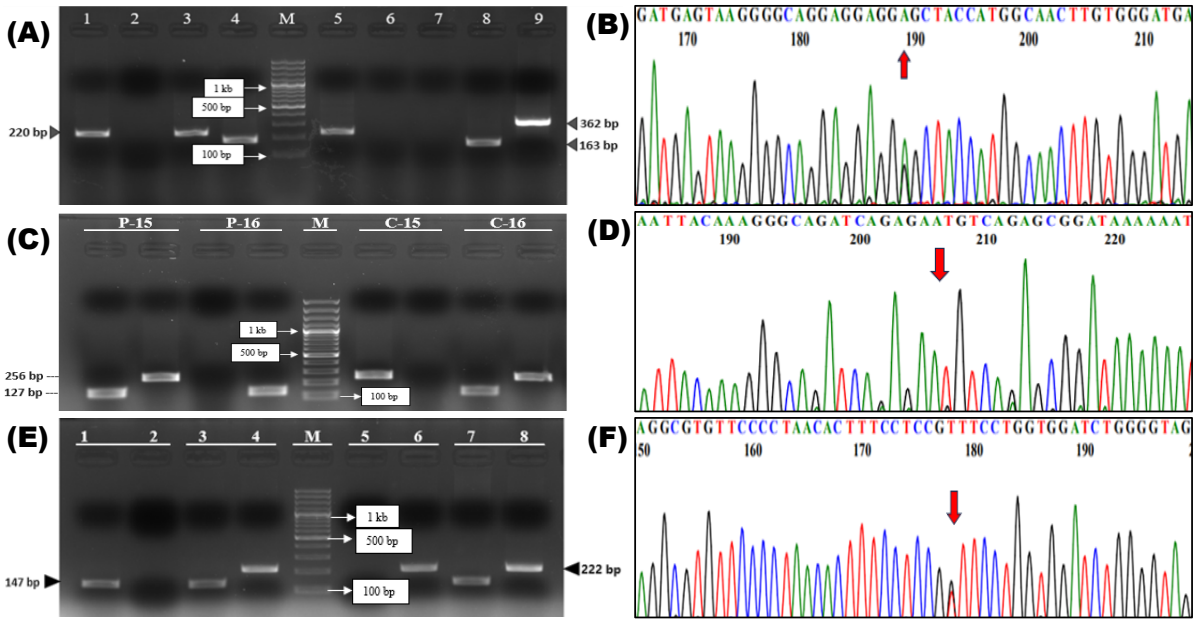

FIGURE 1. Representative image of agarose gel electrophoretogram for the genotyping and sequencing. (A) *MATE2* (*SLC47A2*) rs12943590, G/A polymorphism, G allele 220 bp, A allele 163 bp, wells pair 1-2 GG genotype, pair 3-4 GA genotype, 5-6 GG, 7-8 AA genotype, well 9 outer product 362 bp, M molecular marker 100bp. Well 9 outer PCR product for random sequencing. (B) Sanger sequencing chromatogram for rs12943590 representing AG heterozygous genotype. (C) Agarose gel electrophoretogram for rs11212617 *ATM* allele (A/C genotype), M molecular marker (100 bp), patients' samples represented as P-15 and P-16, control represented as C-15 and C-16, A allele 127 bp, C allele 256 bp, P-15 heterozygous AC, P-16 homozygous CC, C-15 homozygous AA, and C-16 heterozygous. (D) Sanger sequencing chromatogram for rs11212617, intron variant homozygous AA. (E) Agarose gel electrophoretogram for of *OCT2* (*SLC22A2*) rs7757336, Lane 1-2 GG, 3-4 and 7-8 GT, 5-6 TT, M 100 bp molecular marker. (F) Sanger sequencing chromatogram of allele for rs7757336, intron variant, heterozygous TG

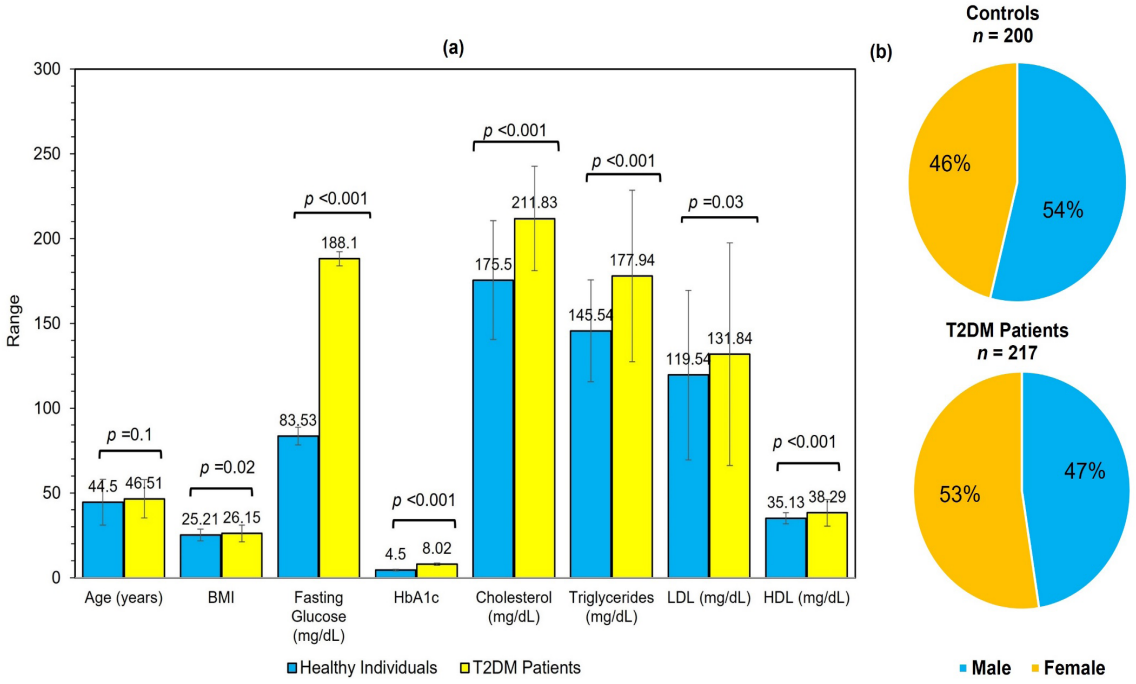

FIGURE 2. Characteristics of healthy individuals and T2DM patients under study. A pair wise comparison has been done with corresponding *p*-value representing statistical significance (a), and demographic profile of controls and T2DM patients (b)

while non-responders were 60. Those patients whose % HbA1c decreased during metformin monotherapy by 0.5% were grouped as responders, while those whose %HbA1c either did not change or even increased by at least 0.5% were called non-responders.

Figure 3 represents a comparative analysis of various biochemical, clinical, and biophysical parameters of both responders and non-responders under study. A suitable baseline was set for both groups. For all parameters, the difference between baseline and after metformin therapy was recorded as mean percent change. Parameters with significant ($p \leq 0.05$) mean percent difference were BMI, HbA1c, FBG, LDL, and TC, while those with non-significant change were age, HDL, and TG. Gender, wise number was 38 males, 22 females in non-responders, while 65 males and 92 females were in the responder group.

ASSOCIATION OF *MATE2* RS12943590, *ATM* RS11212617 AND *OCT2* RS7757336 GENE POLYMORPHISM WITH METFORMIN RESPONSE AND GLYCEMIC CONTROL

The statistical data analysis of genotypes and allele frequency between metformin responders and non-responders was evaluated in terms of their odds ratios (OR) and *p*-values (Table 2). Our data shows that *MATE2* (*SLC47A2*) rs12943590 GA genotype (OR 0.44, CI 95% 0.23-0.86, $p = 0.01$), *OCT2* (*SLC22A2*) rs7757336 GG genotype (OR 5.82, 95% CI 1.40-24.24, $p = 0.01$), G allele (OR 2.21, CI 95% 1.18-4.14, $p = 0.01$) are significantly associated with metformin response or efficacy and thus glucose-lowering effect. On the other hand, we did not find any association of significant nature ($p > 0.05$) for *ATM* rs11212617 (Figure 4).

Different genetic models were evaluated for all the SNPs to affirm their associations with metformin efficacy and glycemic control in this study. A similar trend was observed, as seen in Table 3. In dominant and additive models for rs12943590, significant association (OR 0.47, CI 95% 0.25-0.88, $p = 0.01$) and (OR 0.49, CI 95% 0.2728-0.8887, $p = 0.01$), respectively, have been observed. Moreover, for rs7757336 additive model (OR 2.24, CI 95% 1.1440 to 4.3892, $p = 0.01$) it has been found to have an association with metformin efficacy in metformin responder individuals. There was no association found in any of the genetic models for rs11212617. These results are consistent with the ones observed in Table 2, altogether, to affirm that *MATE2* (*SLC47A2*) rs12943590 GA genotype, *OCT2* (*SLC22A2*) rs7757336 GG genotype, and G allele are significantly associated with metformin efficacy or response in metformin-responsive individuals.

Studies done previously showed that variability in the metformin response is due to gene polymorphism in the metformin transporter genes either present in coding or non-coding regions (Pradana et al. 2024; Zaharenko et al. 2016). In our current work, we investigated three hotspot SNPs or polymorphic loci and their association with metformin response in the T2DM patients of the Khyber Pakhtunkhwa Pakistani population.

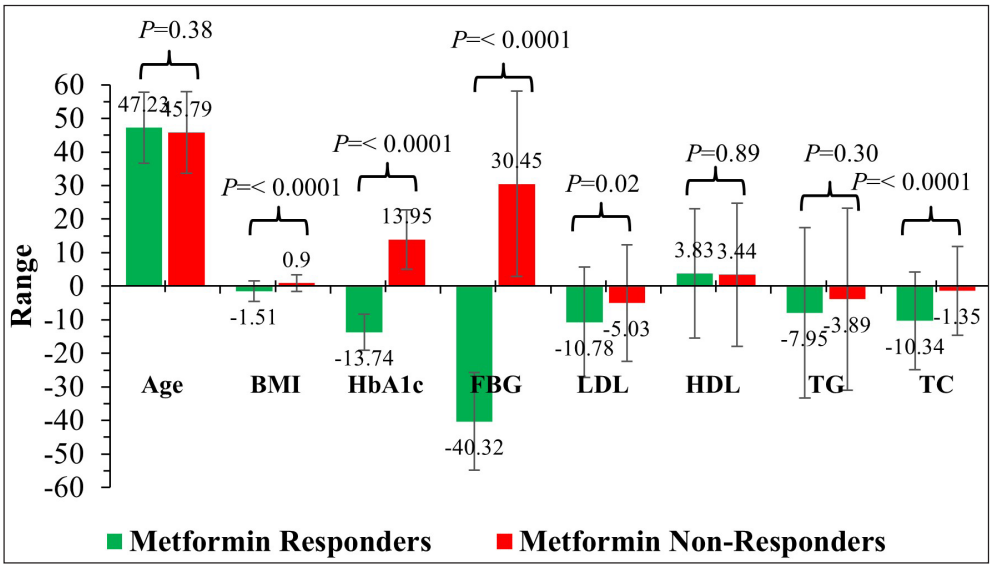

FIGURE 3. Statistical comparative analysis of clinical parameters between metformin responders and non-responders

TABLE 2. Allele and genotype frequency distribution in rs12943590, rs11212617 and rs7757336 in metformin responders and non- responders

	Controls	T2DM	Metformin non-responders	Metformin Responders	OR	CI (95%)	*p*-Value
	n=200 (%)	n=217 (%)	n=60(%)	n=157(%)			
rs12943590							
GG	71 (35.5)	71 (32.71)	27 (45)	44 (28)	Reference		
GA	92 (46)	111 (51.15)	24 (40)	87 (55.41)	0.44	0.23-0.86	*0.01
AA	37 (18.5)	35 (16.12)	9 (15)	26 (16.56)	0.56	0.23-1.38	0.21
Alleles							
G	234 (58)	253 (58.29)	78 (65)	175 (55.73)	Reference		
A	166 (41)	181 (41.70)	42 (35)	139 (44.26)	0.67	0.43-1.04	0.08
rs11212617							
CC	25 (12.5)	30 (13.8)	10 (16.66)	20 (12.7)	Reference		
CA	75 (37.5)	82 (37.78)	21 (35)	61 (38.85)	0.68	0.27-1.70	0.41
AA	100 (50)	105 (48.38)	29 (48.33)	76 (48.4)	0.76	0.31-1.82	0.54
Alleles							
C	119 (29.75)	142 (32.7)	41 (34.16)	101 (32.16)	Reference		
A	281 (70.25)	292 (67.28)	79 (65.83)	213 (67.83)	0.91	0.58-1.42	0.69
rs7757336							
TT	170 (85)	180 (82.94)	46 (76.66)	134 (42.67)	Reference		
TG	23 (11.5)	28 (12.90	8 (13.33)	20 (5.98)	1.16	0.48-2.82	0.73
GG	7 (3.5)	9 (4.14)	6 (10)	3 (0.955)	5.82	1.40-24.24	*0.01
Alleles							
T	362 (90.5)	388 (89.40)	100 (83.33)	288 (91.71)	Reference		
G	38 (9.5)	46 (10.59)	20 (16.66)	26 (8.28)	2.21	1.18-4.14	*0.01

*Statistically significant association ($p \leq 0.05$). OR represent odds ratio, CI confidence interval

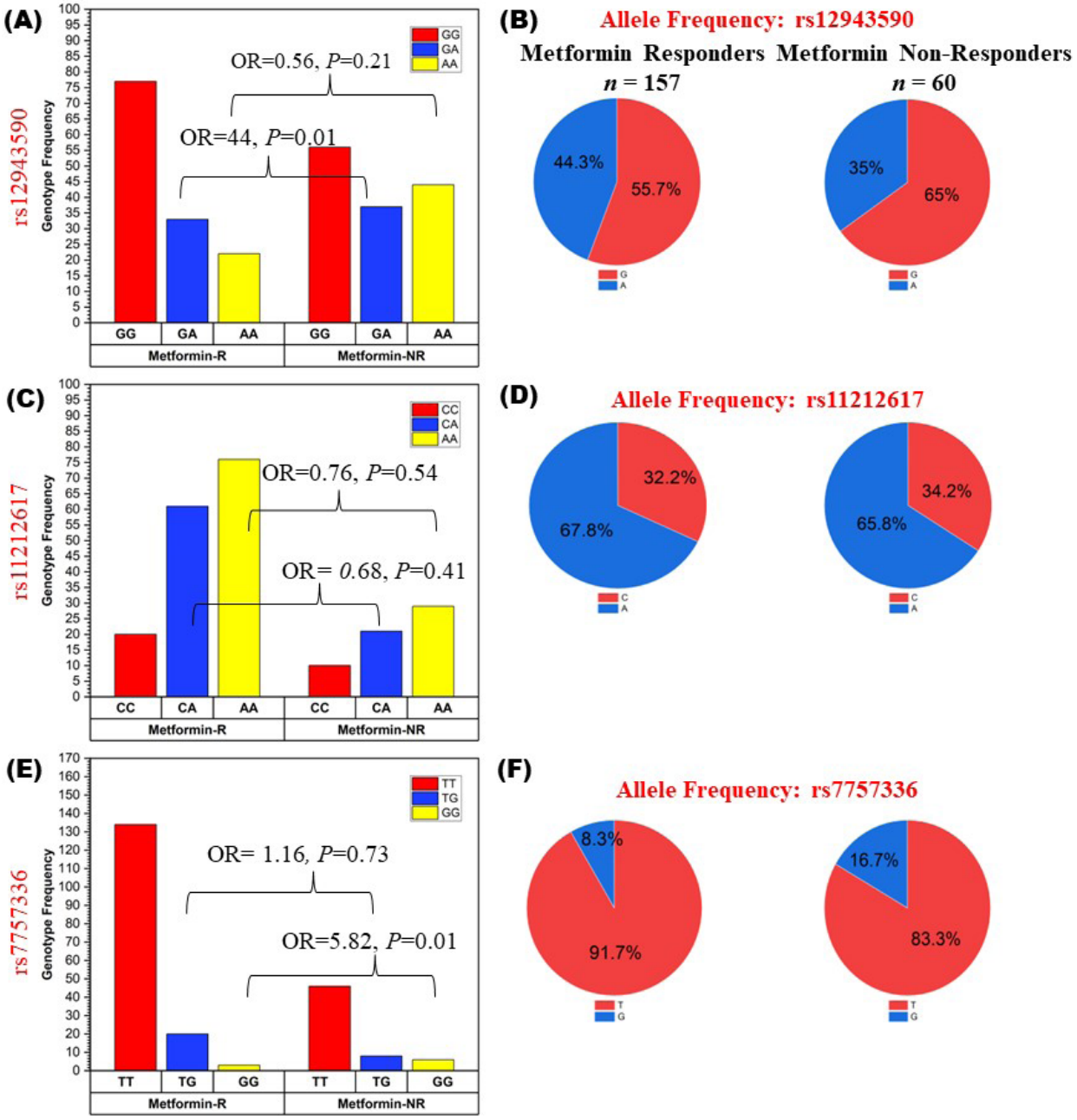

FIGURE 4. Genotype and allele frequency distribution between metformin responders and non-responders for rs12943590, rs11212617 and rs7757336. Respective odd ratios and *p*-values have been shown in a pair wise comparison. On left side genotypes represented in graphical manner as A, C, E while allele frequency on right side in pi-charts as B, D and F

MATE2 the transporter protein present in the renal tubules at the basal domain along the distal and proximal area is involved in metformin transport into urine thus individual variation in its structure or expression may alter metformin pharmacokinetics. The rs12943590 (−130G > A) variant present at the basal promoter region was significantly associated with increased promoter activity. Choi et al. (2011) characterized different variants of SLC47A2 to find out its association with metformin efficacy. In their study, they significantly associated variant (485C > T and 1177G > A) with lower metformin uptake and reduced expression of the protein. Moreover rs12943590 (−130G > A) variant present at the basal promoter region was significantly associated with increased promoter activity. Choi et al. (2011) associated rs12943590 (−130G > A) homozygous condition with weaker response to metformin.

TABLE 3. Evaluation of different genetic models for rs12943590, rs11212617 and rs7757336 in metformin responders and non-responders

Genetic model	Genotype	OR	CI (95%)	*p*-Value
rs12943590				
Dominant	GG Vs GA+AA	0.47	0.25-0.88	*0.01
Recessive	AA Vs GG+GA	0.48	0.2082 to 1.1152	0.08
Over-dominant	GG+AA Vs GA	0.82	0.4525 to 1.5171	0.54
Additive	A Vs GG	0.49	0.2728 to 0.8887	*0.01
rs11212617				
Dominant	CC Vs AC+AA	0.72	0.3198 to 1.6661	0.45
Recessive	AA Vs CC+AC	0.99	0.5498 to 1.8082	0.99
Over-dominant	CC+AA Vs AC	1.18	0.6348 to 2.1937	0.60
Additive	A Vs CC	0.74	0.3327 to 1.6539	0.46
rs7757336				
Dominant	TT Vs TG+GG	1.77	0.8426 to 3.7315	0.13
Recessive	GG Vs TT+TG	2.00	0.4756 to 8.4106	0.34
Over-dominant	TT+GG Vs TG	0.94	0.3936 to 2.2875	0.90
Additive	G Vs TT	2.24	1.1440 to 4.3892	*0.01

*Statistically significant association ($p \leq 0.05$). OR represent odds ratio, CI confidence interval

For a comprehensive, holistic approach and insight into the pharmacogenetics of metformin, 3 different full-scale GWAS have been carried out. The first GWAS was performed in 2011 in a Genetics of Diabetes Audit and Research Tayside (GoDART) cohort of European ancestry (GoDARTS et al. 2011). A total sample size of 1024 Scottish T2DM patients was taken. Fourteen important SNPs were highlighted in this study. Allele C of rs11212617 was found to be associated with metformin response in reducing HbA1c level (Zhou et al. 2016). rs11212617 was also associated in the different sample set with a significant *p*-value. The *ATM* gene has been considered to be an important gene here, along with other related genes in the same block (Harries et al. 2011). SNP rs11212617 has been associated with metformin response in independent studies in different populations like Chinese Hans and Saudi Arabian (Altall et al. 2019; Zhou et al. 2014). In another two studies, no significant difference has been found between T2DM individuals as compared to healthy ones with SNP rs11212617 (Shokri et al. 2016; Vilvanathan et al. 2014). The inconsistency between these results may be due to differences in the ethnicities, sample size, and individuals' circumstances or approaches used for the selection of sample individuals (Hakim et al. 2024; Naesa & Joujeh 2024; Peng et al. 2023). OCT2, which is organic cation transporter 2, located at the basolateral side of the renal tubule, carries out the transportation or excretion of metformin along with MATE1 and MATE2 transporters into urine (Ito et al. 2012; Liang & Giacomini 2017).

The rs7757336 SNP locus is present between coding genes *OCT2* and *OCT3*. Previous work has shown that this very SNP is significantly associated with metformin inefficacy in certain T2DM individuals. It was also shown that the minor allele of rs7757336 was assumed to be involved in low response to metformin, as the plasma concentration of metformin was assessed to be low, depicted from the area under the curve for plasma concentration of metformin (Zaharenko et al. 2016).

Our results data showed that *MATE2* (*SLC47A2*) rs12943590 GA genotype (OR 0.44, CI 95% 0.23-0.86, $p = 0.017$) and *OCT2 (SLC22A2)* rs7757336 GG genotype (OR 5.82, 95% CI 1.40-24.24, $p = 0.015$), G allele (OR 2.21, CI 95% 1.18-4.14, $p = 0.012$) are significantly associated with metformin efficacy and glycemic response in newly diagnosed and drug naive T2DM individuals, while no significant association ($p > 0.05$) was observed for *ATM* rs11212617. All polymorphisms in the current study are noncoding and could be in linkage disequilibrium with other causative SNPs whose altered nature influences the expression levels of target proteins and hence the transport activity of metformin. There are various limitations to the study, as during follow up the patients were also advised to modify their lifestyle, as in routine clinical practice, all measures for lifestyle modifications are advised. Patients' physical exercise and a healthy low glycemic diet may interfere with the results of metformin-induced decreased HbA1c, therefore, independent study in healthy individuals may be carried out for these SNPs to ascertain its more specific role. As the data obtained is from a population

of about forty million with a cumulative sample size of 417 individuals and with a confined ethnicity of the area therefore a more comprehensive study in various other larger populations with a larger sample size may be carried out. Genome-wide association studies nowadays is a more comprehensive strategy that can be utilized in a meaningful way. As our study is based on statistical genetic associations which reduces the time and energy towards targeted functional studies, therefore a more precise functional characterization can be carried out to pinpoint the functional consequences of these genetic variations for future precision medicine.

CONCLUSION

In summary, we conclude that *MATE2* (*SLC47A2*) rs12943590 heterozygous GA genotype, *OCT2* (*SLC22A2*) rs7757336 homozygous GG genotype, and G allele are significantly associated with metformin response, efficacy, and glucose-lowering effect in terms of HbA1c in newly diagnosed drug-naive T2DM patients. These associations are assumed to positively alter the pharmacokinetics of metformin in the subject patients either by a mechanism involving these SNPs only or in a linkage disequilibrium manner. These significant associations may lead to the designing of tailored precision medicine in the future, generally and in the subject ethnicity, particularly for T2DM patients. No significant association was observed for *ATM* rs11212617, or in other words, *ATM* rs11212617 is related to metformin inefficiency.

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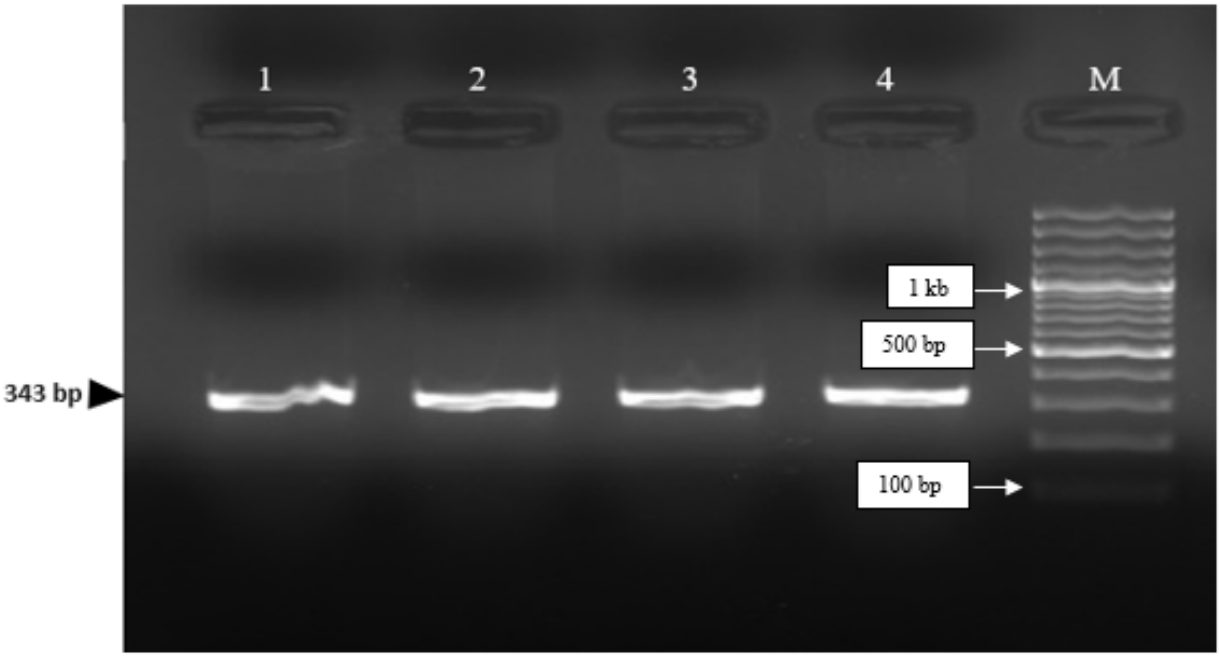

FIGURE S1. Representative image for the outer PCR product rs11212617 for sequencing, lane 1-4 sample wells, M molecular marker 100 bp, outer product 343 bp

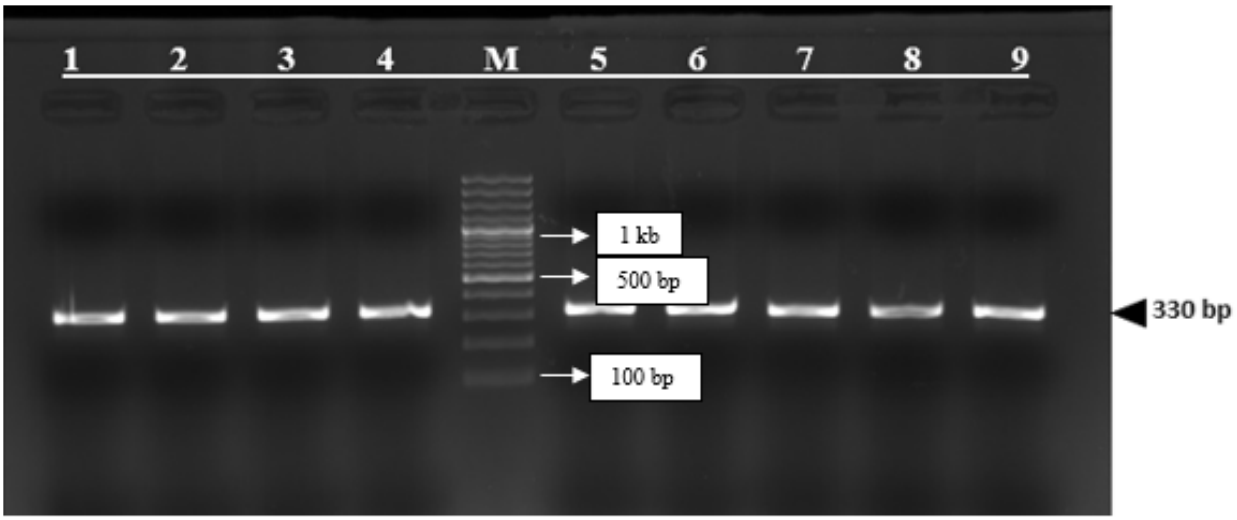

FIGURE S2. Representative image for the PCR outer product of *OCT2* (*SLC22A2*) rs7757336 for sequencing, Lane 1-9 PCR outer product, M molecular marker 100 bp. Outer product 330 bp

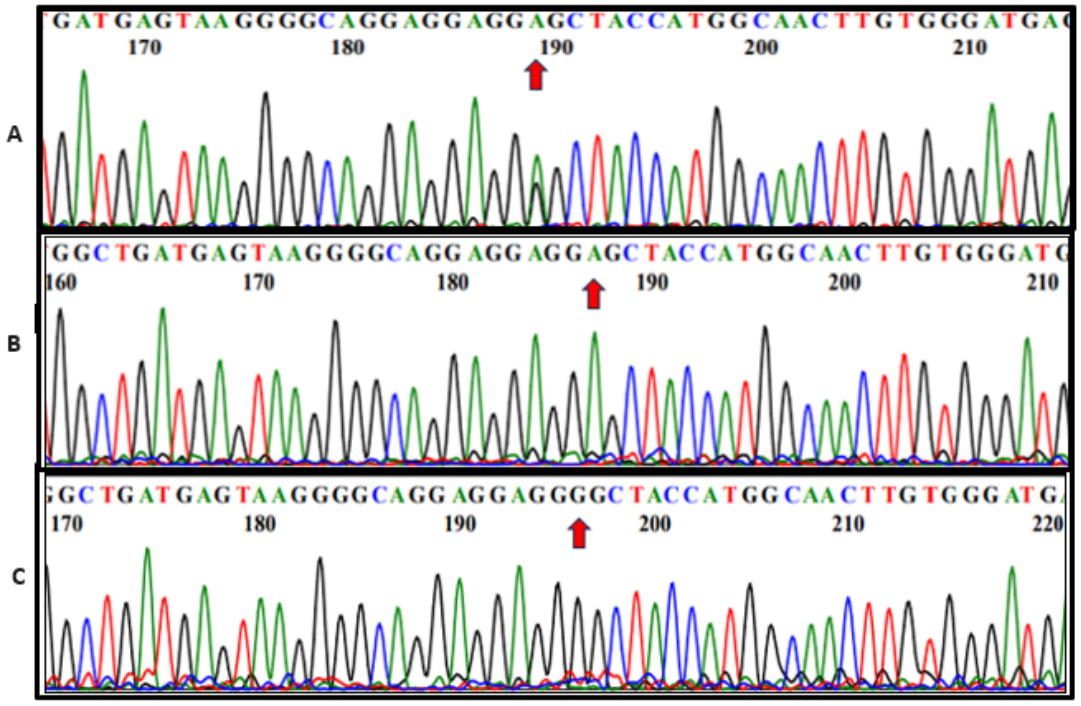

FIGURE S3. Sanger sequencing chromatograms of alleles for rs12943590, 5 prime UTR variant, (A) Heterozygous GA, (B) Homozygous AA and (C) Homozygous GG

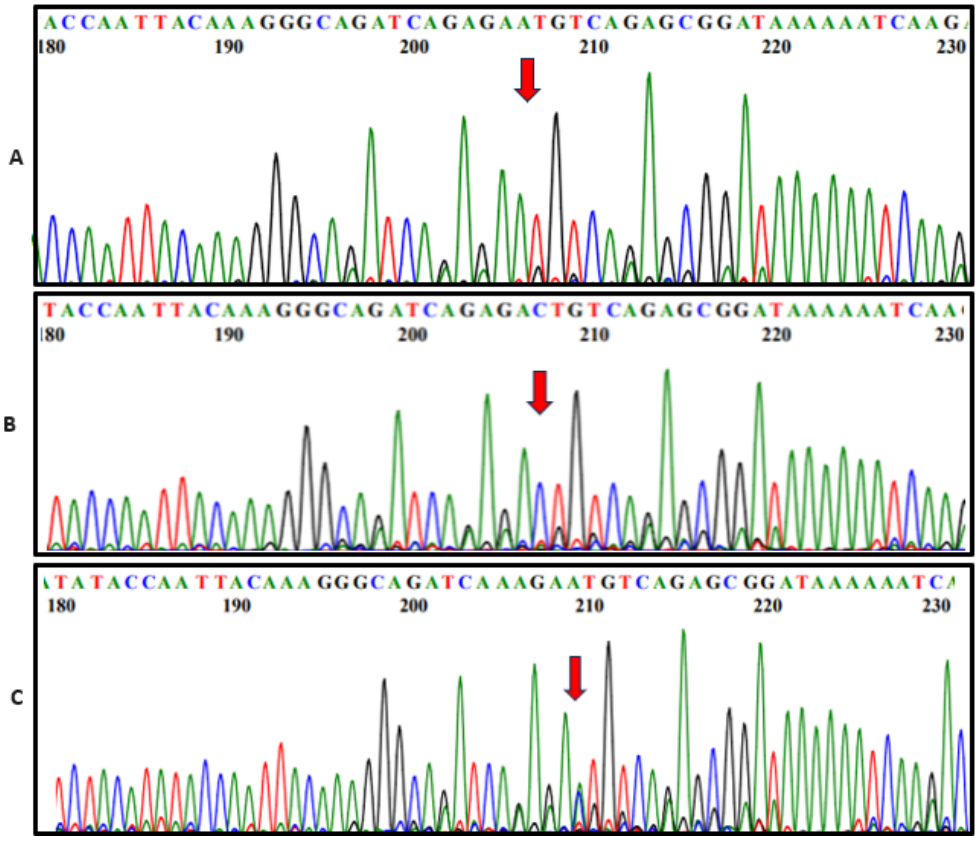

FIGURE S4. Sanger sequencing chromatograms of alleles for rs11212617, intron variant, (A) Homozygous AA, (B) Homozygous CC and (C) Heterozygous AC

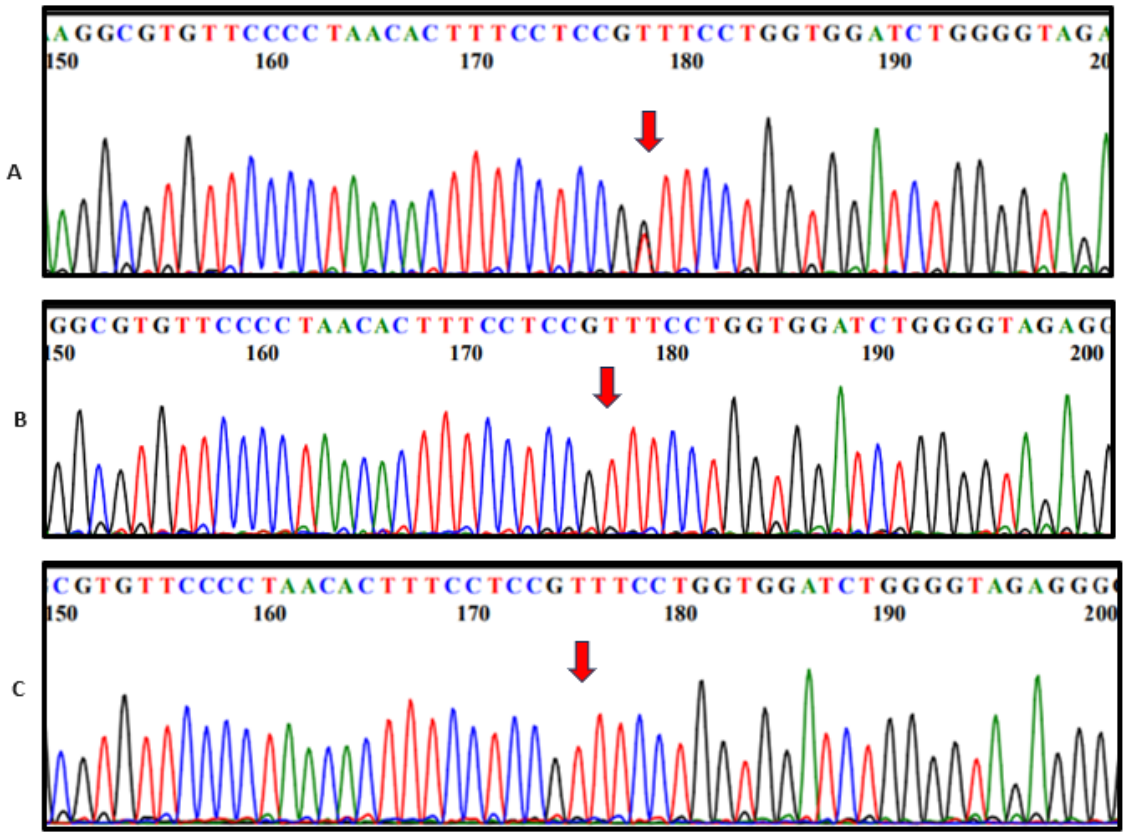

FIGURE S5. Sanger sequencing chromatograms of alleles for rs7757336, intron variant, (A) Heterozygous TG, (B) Homozygous TT and (C) Homozygous TT